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IODINE DEFICIENCY IN A COMMONLY USED STOCK DIET

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It was shown in previous work by the authors ('35) that the addition of 1% cod liver oil to a supposedly complete diet produced thyroid glands in female rats which were significantly smaller than those of their litter-mate controls. Since it is known that the thyroid gland is sensitive to iodine, the possibility that the iodine present in cod liver oil was responsible for the changes suggested itself. Dr. Roe E. Remington, in a personal communication, suggested, as a result of his observations, that the changes might possibly be due to the addition of iodine (in cod liver oil) to an iodine deficient diet.

The present work was carried on to find out whether the change could be found in males; whether time had any influence upon the change; and whether the change was due to the iodine in the administered cod liver oil.

MATERIALS AND METHODS

Male Wistar albino rats were used. The animals were weaned at 3 weeks of age and immediately placed on the diets. The basal diet consisted of: casein, 15%; whole milk powder, 10%; sodium chloride, 0.8%; calcium carbonate, 1.5%; butter (unsalted), 5.2%; and whole ground wheat, 67.5%. The mixture was prepared fresh at least every other day.

The animals were divided into three series. Series A consisted of twenty-five males which received the basal diet plus

2% pure cod liver oil (U.S.P.). Twenty-five litter mates received only the basal diet. The animals of this group remained on the diet until 4 months of age and were then autopsied.

In series B twenty-six males received the basal diet plus 2% cod liver oil. Twenty-six litter mates were sustained on the basal diet. The animals of this group were kept on the diet until the age of 3 months and then killed.

Series C consisted of five groups of male rats. Twenty-five were sustained on the basal diet; twenty-six had 1% oil added to their diet; twenty-six received 2% oil; twenty-six received 0.0184% potassium iodide, and twenty-six animals received 0.0184% potassium iodide plus 2% cod liver oil in addition to the basal diet described above. The rats of this series were autopsied at 2 months of age. The source of the iodine was Morton's iodized salt which the manufacturers state contains 0.023% of potassium iodide.

The progress of each animal was carefully watched, and each was weighed weekly. At the time of autopsy in the 4-month and 3-month series, the body and tail lengths were measured in addition to the weights of the head, suprarenal glands, hypophysis, thyroid, thymus and testes. In the 2-month series the lengths of the body and tail were measured as well as the weights of the head and the thyroid gland. The organs removed were fixed in Bouin's fluid. Sections of the thyroid glands of the 2-month series were stained with hematoxylin and eosin.

A comparison of the results from the various groups of animals was made, using modern biometrical methods. As is customary the difference between the two means divided by the probable error of the difference is spoken of as the significance ratio in this paper.

OBSERVATIONS

Thyroid gland

Series A. The mean thyroid weight (table 1) of the rats without oil in their diet was 0.02620 gm. The average weight

of the thyroid of those animals which received oil was 0.02025 gm. The 22.71% decrease in the oil group was definitely significant. The significance ratio for the difference was 7.88.

Series B. The thyroid gland of the animals in this group (table 2) showed a definite weight change. The mean value

TABLE 1
Statistical summary of results of the 4-month group.
(Measurements in grams and centimeters)

	MEAN		DIFFERENCE BETWEEN MEANS	SIGNIFI- CANCE RATIO
	Oil	No oil		
Hypophysis	0.00868±0.00015	0.00838±0.00012	0.00030±0.00019	1.59
Thyroid	0.02025±0.00044	0.02620±0.00061	0.00595±0.00075	7.88
Thymus	0.53233±0.01790	0.52694±0.01819	0.00539±0.02552	0.21
Suprarenals	0.02827±0.00051	0.02742±0.00045	0.00085±0.00068	1.25
Testes	2.9447 ±0.0494	3.1237 ±0.0462	0.1790 ±0.0676	2.65
Body weight	300.8 ±5.2	290.8 ±5.1	10.04 ±7.28	1.38
Head weight	23.08 ±0.30	22.50 ±0.25	0.58 ±0.39	1.47
Body length	22.32 ±0.11	22.18 ±0.11	0.14 ±0.15	0.91
Tail length	20.68 ±0.15	20.51 ±0.17	0.16 ±0.23	0.71

TABLE 2
Statistical summary of results of the 3-month group.
(Measurements in grams and centimeters)

	MEAN		DIFFERENCE BETWEEN MEANS	SIGNIFI- CANCE RATIO
	Oil	No oil		
Hypophysis	0.00824±0.00012	0.00826±0.00015	0.00002±0.00019	0.10
Thyroid	0.02010±0.00053	0.02596±0.00052	0.00586±0.00074	7.93
Thymus	0.6225 ±0.01738	0.68070±0.01829	0.05820±0.02523	2.31
Suprarenals	0.02718±0.00036	0.02784±0.00050	0.00065±0.00062	1.06
Testes	2.99259±0.03221	3.15876±0.06913	0.16617±0.07626	2.18
Body weight	274.2 ±3.7698	273.0 ±4.7011	1.2 ±6.026	0.20
Head weight	21.89 ±0.1970	21.69 ±0.2835	0.20 ±0.3452	0.58
Body length	22.08 ±0.1140	21.81 ±0.1219	0.27 ±0.1669	1.62
Tail length	20.39 ±0.1016	20.29 ±0.1231	0.10 ±0.1596	0.63

for the no-oil animals was 0.02596 gm. while that for the rats with 2% oil was 0.02010 gm. The decrease in the oil group amounted to 22.57%. The difference was significant, the significance ratio being 7.93.

Series C. The mean autopsy weight (table 3) of the several groups of this series were: no-oil, 0.01646 gm.; KI-no oil,

0.01271 gm.; 1% oil, 0.01464 gm.; 2% oil, 0.01427 gm.; and KI-2% oil, 0.01262 gm. The no-oil glands were larger than the other glands. The KI-no oil glands were decreased 22.80% when compared to the no-oil group; the 1% oil group, 11.04%; the 2% oil group, 13.31%; and the KI-2% oil group, 23.31%. All of these decreases were significant as shown by the following significance ratios: KI-no oil, 7.46; 1% oil, 3.51; 2% oil, 4.14; and KI-2% oil, 8.24 (no-oil group used for comparison).

TABLE 3
Means of 2-month group. (Measurements in grams and centimeters)

	NO OIL	KI-NO OIL	1% OIL	2% OIL	KI-2% OIL
Thyroid	0.01646 ± 0.00038	0.01271 ± 0.00033	0.01464 ± 0.00035	0.01427 ± 0.00037	0.01262 ± 0.00027
Body weight	173.48 ± 3.37	183.92 ± 4.11	186.42 ± 4.07	185.62 ± 3.42	179.38 ± 4.18
Head weight	15.90 ± 0.23	16.26 ± 0.27	16.26 ± 0.26	16.24 ± 0.25	15.90 ± 0.28
Body length	19.29 ± 0.11	19.50 ± 0.12	19.48 ± 0.13	19.43 ± 0.11	19.27 ± 0.14
Tail length	16.86 ± 0.12	17.30 ± 0.14	17.37 ± 0.14	17.11 ± 0.12	17.13 ± 0.14

TABLE 4
Significance ratios of 2-month group. (No oil compared with other groups)

	KI-NO OIL	1% OIL	2% OIL	KI-2% OIL
Thyroid	7.46	3.51	4.14	8.24
Body weight	1.96	2.45	2.53	1.10
Head weight	1.01	1.04	1.01	0.01
Body length	1.27	1.14	0.90	0.13
Tail length	2.40	2.74	1.49	1.48

The histological structure of the thyroid was studied in six animals of each group. No changes were noted.

Other measurements

The average measurements of body weight, head weight, body length, tail length, hypophysis, thymus, suprarenal glands and testes showed no differences between the tests and controls which were of certain significance in any of the three series. The average measurements are given in the tables and since the results are negative no further discussion is needed.

INCIDENCE OF INFECTIONS

All animals were examined carefully for signs of infections. Very few pathological changes were noted. There was no observed difference in the amount of respiratory or middle ear infection in any of the series studied.

DISCUSSION

Strikingly apparent was the effect of adding cod liver oil to the basal diet used in the present experiment. The thyroid glands of male rats, sustained on diets to which 1% cod liver oil and 2% cod liver oil had been added, at 4 months, 3 months, and 2 months of age were significantly smaller than those of animals without oil in the diet. The same phenomenon has been observed in the case of female rats at 4 months of age (Freudenberger and Clausen, '35). Interesting enough was the fact that no significant changes were found in any of the other structures studied in the 4-month and 3-month series. On this basis the gonads, hypophysis, thymus, and suprarenal glands were not studied in the 2-month series.

Naturally, the question arose as to which constituent, or constituents, of the cod liver oil was responsible for these definite and consistent weight changes in the thyroid gland with no significant alteration in any of the other structures studied. The adequacy of the diet together with the close parallelism of the various groups of animals both as to body growth and weights of the several organs (except thyroid), most certainly spoke against the possibility of any form of an avitaminosis.

The sensitivity of the thyroid to the iodine intake is an established fact. This seemed to be by far the most likely constituent of the cod liver oil responsible for the changes. The effects of iodine on the thyroid gland have been studied by many investigators. Hayden, Wenner and Rucker ('23-'24) found thyroid enlargement in rats on a diet containing a small amount of iodine (9 to 10 parts per billion). In the same period McClendon and Williams made similar observations. By the addition of iodine, these authors found that

smaller thyroid glands were obtained. Marine ('24) maintained that simple goiter was caused by a deficiency of iodine. Tanabe ('25) confirmed Marine with results obtained from the rat. Krause and Monroe ('30) found that the addition of iodine to diets low in this constituent resulted in smaller thyroid glands in rats. Thompson ('32) reported enlargement of thyroids in rats on a rachitogenic diet. The addition of iodine, not vitamin D, produced smaller glands. Levine, Remington and von Kolnitz ('33) worked out a range of iodine dosage which was proved to be very sensitive. The range was between 0.14γ and 0.59γ ($\gamma=0.001$ mg.) daily. Small variations in the amount of iodine within this range produced marked changes in the thyroid glands. The authors came to the conclusion that the minimum daily intake of iodine to prevent goiter in the rat was from 1 to 2γ .

Cod liver oil as a source of iodine is without dispute. Holmes and Remington ('35), reporting on the iodine content of twenty representative samples of American cod liver oil, found the iodine content to average 8640 parts per billion parts of cod liver oil.

The evidence, from the data obtained with the 4-month and 3-month series, was very suggestive that the basal diet fed the animals was inadequate in iodine. That is, the rats received iodine which perhaps fell within the critical range of iodine intake mentioned above (less than $\frac{1}{2}\gamma$ daily).

In the 2-month group iodine in the form of potassium iodide was added to the basal diet to find out whether the glands could be reduced in size, as was the case with the administration of cod liver oil. The iodized salt was used in place of the normal salt in the basal diet. The amount of potassium iodide in the salt was stated as 0.023%. Since the amount of salt in the basal diet was 0.8%, the amount of potassium iodide was calculated to be 0.0184%. This value would give each animal approximately 14γ of iodine daily (assuming each animal ate 10 gm. of food daily). Such an iodine intake is far in excess of the physiological requirement for the rat.

The results showed that the administration of potassium iodide to the basal diet produced a much greater change in the weight of the thyroid gland than was found with the administration of either 1 or 2% of cod liver oil. It would seem, therefore, that the iodine in the cod liver oil was not available to the organism in sufficient quantities to render the basal diet completely antigoitrogenic (did not remove the iodine intake from the critical range cited above). Thus, iodine in the form of potassium iodide was more effective in preventing goiter than the iodine in the cod liver oil.

An unexplainable bit of information was the fact that the 2% oil had practically no more effect than did the 1% oil. Certainly, one would think that if 1% cod liver oil was not sufficient to bring the intake of iodine out of the critical range, 2% oil would produce a change greater than that produced by the 1% oil. Such was not the case. Two per cent cod liver oil did not have the magnitude of influence in the 2-month series that it did in the 3- and 4-month series. This fact may or may not have significance.

Remington, Remington and Welch ('37) reported normal thyroid weights which were lower than those reported in the literature with the exception of those of McCarrison ('30). They called attention to the importance of an adequate amount of iodine in the diet and suggested that a stock diet such as that used by us was probably deficient in iodine. Our results would tend to confirm their contentions.

SUMMARY AND CONCLUSIONS

A total of 231 male Wistar albino rats was divided into three series. The animals of all series were placed on the various diets at 3 weeks of age. Series A consisted of twenty-five rats fed a well-balanced basal diet plus 2% cod liver oil, and twenty-five rats fed on the basal diet for controls. The animals were killed and autopsied at 4 months of age. Series B was composed of twenty-six test rats and twenty-six controls treated exactly as those of series A, except that the animals were killed and autopsied at 3 months of age. In series

C there were five groups of rats. Twenty-five were sustained on the basal diet; twenty-six had 1% oil added to the diet; twenty-six received 2% oil; twenty-six had 0.0184% potassium iodide added to the basal diet; and twenty-six animals received the same amount of potassium iodide plus 2% cod liver oil. The rats of this group were autopsied at 2 months of age.

No significant difference in any of the series was found in body weight, body length, tail length, incidence of infection, or in the weights of the head, suprarenal glands, hypophysis, thymus, or testes.

The thyroid gland was significantly smaller in all groups of animals which received either cod liver oil or potassium iodide, or both. The change was most marked in those animals which had potassium iodide present in their diets.

The conclusion was reached that the stock diet fed the animals of this colony is iodine deficient. This condition was compensated for in part by the addition of cod liver oil. Potassium iodide added to the stock diet was more efficacious in obviating the condition. There was apparently no difference between the sexes. There seemed to be a greater change in thyroid weight in the series of animals sustained on the diets for the longer periods of time, namely, 3 and 4 months.

Lastly, iodized salt should be substituted for ordinary salt in basal diets, at least in goitrogenic belts, in order to supply the thyroid gland (and the organism) with the physiological requirement of iodine. The result will be a lower normal value for thyroid gland weights.

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VITAMIN G AND SYNTHETIC RIBOFLAVIN¹

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ONE FIGURE

(Received for publication July 14, 1937)

Vitamin G (B₂) previous to 1933 was defined (American Society of Biological Chemists, '29) as the heat-stable water soluble dietary factor necessary for maintenance and growth in the rat. It was generally assumed the same factor prevented the occurrence of a dermatitis which frequently accompanied this deficiency (Goldberger and Lillie, '26; Sherman and Sandel, '31). The identity of vitamin G to the P-P factor of Goldberger was not clear, but the facts at that time made it seem probable that they were the same (Goldberger, Wheeler, Lillie and Rogers, '26; Sherman and Smith, '31). The evidence now indicates that several factors were involved in this deficiency as previously defined (Gyorgy, '35; Harris, '35; Koehn and Elvehjem, '36). It is therefore important to be able to interpret the earlier rat tests in terms of newer knowledge.

The Sherman-Bourquin diet ('31) which contains an alcoholic extract of ground whole wheat as a source of vitamin B₁ and other possible essentials in this group was very widely used for vitamin G determinations. It is the object of this paper to show that this method was a test for riboflavin and to report the rat growth response to synthetic riboflavin of known purity.

¹ Aided by a grant from the Milton Fund to the University Committee on Research in Dental Medicine.

Using a diet in which a yeast preparation supplied the water-soluble accessory factors other than riboflavin Karrer ('35) found 5.5 gm. weekly growth when 3 γ daily of riboflavin were fed. On a similar diet Euler and others ('34) reports 7.7 gm. weekly response to 5 γ daily and Kuhn ('35) obtained 10.2 gm. growth with 10 γ daily.

Kuhn ('34) also reported that it was necessary to add a 'B₄ concentrate' to the Bourquin diet in order to obtain a growth response to riboflavin. On the contrary, several papers (Bisby and Sherman, '35; Ansbacher, Supplee and Bender, '36; Copping, '36) have indicated that this method probably was an assay of riboflavin. However, the evidence has not been conclusive because the purity of the riboflavin preparations was not established. The potency of preparations from different laboratories vary considerably, no doubt because many of them to which the term lactoflavin (riboflavin) has been applied are only concentrates. To obtain a yellow powder or a crystalline material is no criterion of purity. Experience has shown that such preparations may be far from pure compounds.

The riboflavin used in these experiments was synthesized by a method similar to that used by Karrer ('35). It is a bright orange yellow substance which easily crystallizes from water and although the substance after the first recrystallization was homogeneous, it was necessary to recrystallize it many times before a constant melting point was reached. The pure substance has a melting point of 293°C.² Nitrogen 14.80 (calculated 14.88). The acetyl derivative was prepared (M.P. 239°C.) and reconverted to riboflavin, which had the same properties as the original.

The tests were carried out in every detail as previously described (Bourquin and Sherman, '31). Rats were transferred from a wheat and milk diet at 28 days (45 to 50 gm.) to a basal diet composed of purified casein, 18 parts, salt mixture

² The substance slowly decomposes above 275° and therefore the melting point by the usual method is not a good constant. By the use of a block, the instantaneous melting point can serve as a useful criteria of purity.

4 parts, butter fat 8 parts, cod liver oil 2 parts, and cornstarch 68 parts, a part of which carried the alcoholic extract of 500 gm. of whole wheat (hard, red) for each kilo of diet.

The rats showed slight weight gains for about 2 weeks, after which time their weights became constant. The supplement of pure riboflavin was started on the fifth week. An accurately

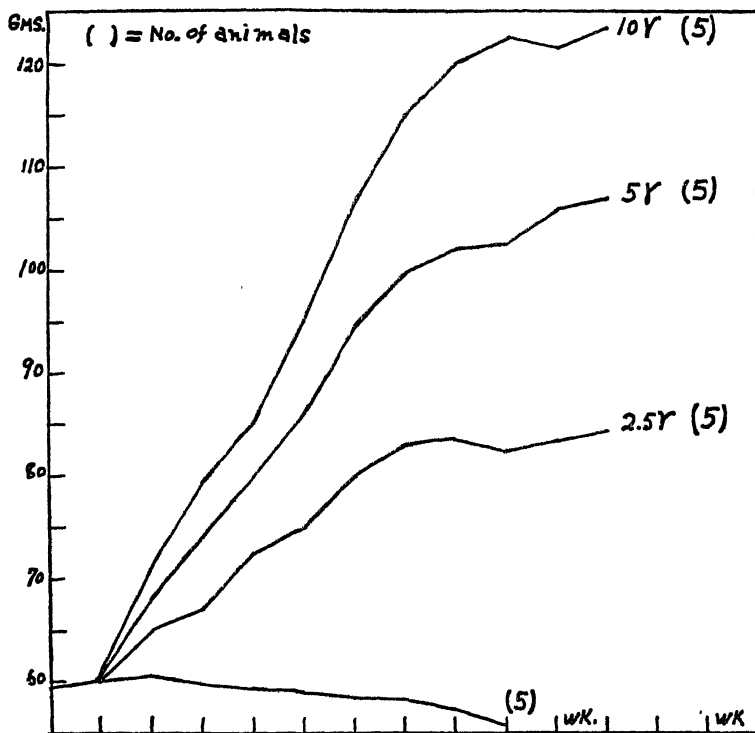


Fig. 1 The average growth of rats receiving the Sherman-Bourquin vitamin G diet plus variable amounts of pure synthetic riboflavin.

prepared solution was fed six times weekly by means of a small graduated pipette. This substance is bitter and it is therefore necessary to be certain that the complete dose is consumed.

Weight response was prompt, uniform and nearly proportional to the levels fed. After 6 weeks the weights of the animals on the lower level remained about constant. A similar

decrease in growth rate occurred on the higher levels a week or so later. At least a part of this is due to the larger size of the animal because an increased supplement of riboflavin will result in resumption of growth. However, the ratio of weight gain to riboflavin beyond this period indicates that probably some other dietary essential is becoming the limiting factor for growth. Such animals may be kept for many weeks without the development of any apparent pathology.

Although the weight increments for increased levels of riboflavin are not so uniform as those of Sherman and Bourquin, this is no doubt due in part to the small number of animals used and in part to growth rates larger than those recommended for this test. It would be desirable to have more data at riboflavin levels between 2 γ and 4 γ . However, it seems reasonable to conclude that this method is a test for riboflavin and calculations based on a 6-week test period indicate that a Sherman Bourquin unit (3 gm. weekly growth) is 2.0 γ to 2.5 γ riboflavin, or there are 400,000 to 500,000 such units per gram of riboflavin.

SUMMARY

The Sherman-Bourquin method of estimation of vitamin G is a test for riboflavin. One unit is equivalent to 2.0 γ to 2.5 γ of riboflavin.

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EFFECT OF DIETS CONTAINING FATS OF VARIOUS DEGREES OF UNSATURATION ON THE SERUM LIPIDS IN RATS ¹

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The investigations of various observers (Henriques and Hansen, '01, '02; Ellis and Isbell, '26; Anderson and Mendel, '28) indicate that the character of depot fat is dependent upon the nature of the diet. The relationship of the diet to blood lipids, on the other hand, has been studied relatively little. Recent reports by Hansen and Burr ('33), Williams and Maynard ('34), Hansen, Wilson and Williams ('36), Hansen and Brown ('37), Brown, Hansen, McQuarrie and Burr ('37) indicate that the blood lipids tend to be less unsaturated when the animal organism is restricted to a low fat regimen. Hansen ('33) first observed the effect of dietary fat on serum lipids when he obtained an increase in the iodine number of the serum fatty acids of certain eczematous individuals following the administration of linseed and corn oil. Similarly Hansen, Wilson and Williams ('36) working with dogs found the iodine numbers of the isolated fatty acids of the serum lipids varied in the same direction as the iodine number of the dietary fats. This is a report of an earlier study in which rats were fed a group of common commercial fats possessing a wide range of iodine numbers.

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The same plan of study and chemical methods were used as in the previous study of this series (Hansen and Brown, '37). Fats were fed at 20% levels, coconut oil, olive oil, lard, Crisco (hydrogenated cottonseed oil), corn oil and linseed oil being used. The average iodine numbers of the various dietary fats as determined are found in table 2. Four or five individual animals were used for each type of food fat. The serum lipids were determined on two or three individual animals of each group and in addition pooled samples were made from two to three animals on the same type of diet. In the large samples it was possible to make duplicate determinations throughout.

The results of the study are presented in table 1.

DISCUSSION

Although the number of animals in each case is few, there is a surprising uniformity in the values observed. The results obtained in the cases of pooled samples of blood from several animals where all the determinations were done in duplicate are in close agreement with that of the average of the group. The iodine numbers of the serum lipids are highest in the groups fed on linseed and corn oil, which substances have the greatest degree of unsaturation. The intermediate values as found in the groups fed on lard and Crisco are consistent with the lesser degree of unsaturation of these substances. The low value for the coconut oil group is in keeping with the saturated condition of this fat. The values obtained for the iodine numbers of the serum lipids for the animals fed the olive oil are rather unexpected in view of the fact that the leaf fat-fatty acids possessed an iodine number of 81 while those of the lard and Crisco rats were 73 and 70, respectively.

As shown in table 1 there is an inverse relationship between the level of the total lipids and their degree of unsaturation. The rats fed on the linseed oil diet have small quantities of total lipids while the coconut oil animals have large amounts of total lipids. The most marked difference is found in the values of the total fatty acids, the same general relationship holding as for the total lipids. The cholesterol values tend

TABLE 1

Serum lipids of rats fed on diets containing fats of various degrees of unsaturation

DIETARY FAT	COLONY NO.	IODINE ABSORBED, MG./100 CC.	CHOLESTEROL, MG. %	TOTAL FATTY ACIDS, MG. %	TOTAL LIPIDS, MG. %	IODINE NUMBERS OF TOTAL LIPIDS
Linseed oil	489	463	72	264	336	138
	492					
	6					
	494	461	103	221	324	142
	490	485	86	279	365	133
	478	332	63	177	240	137
	Average	435	81	235.2	316.2	137.5
Corn oil	476	519	89	306	395	132
	493					
	498					
	499	518	113	254	367	141
	497	506	102	302	404	125
	477	442	75	253	328	135
	Average	496.2	94.8	279	373.5	135
Lard	21	588	81	382	463	127
	15					
	17					
	18	518	105	274	379	136
	19	614	95	437	532	115
	2	549	92	357	447	123
	Average	567	93.2	362.5	455.2	125
Crisco	3	547	81	395	476	115
	4					
	5					
	479	508	99	254	353	144
	1	548	109	351	460	119
	Average	534.3	96.3	333.3	430	126
Olive oil	462	495	88	342	430	115
	467					
	468					
	466	630	97	386	483	130
	Average	542.7	94	355	449	120
Coconut oil	5	513	106	352	457	112
	6					
	11					
	4	573	112	367	479	119
	3	490	89	434	523	94
	Average	539.5	106.5	383.2	490	110

to remain more constant, although they are also lowest in the linseed group and highest in the coconut group. The exact significance of this inverse relationship is not evident; however, it suggests the possibility that the fatty acids of the serum tend to maintain a rather uniform composition as to the number of unsaturated bonds present.

The degree of unsaturation of the serum lipids is definitely less in animals reared on the low fat ration (Hansen and Brown, '37) than in rats given fat in their diets. The average iodine number of the depot fat of rats maintained on the low fat regimen was found to be 66, whereas in the animals fed coconut oil, the average iodine number of the body fat was 54.

TABLE 2

Average iodine numbers of the various dietary fats as compared with the depot fat and the serum lipids of rats

TYPE OF FAT	AVERAGE IODINE NUMBER		SERUM LIPIDS
	Dietary	Depot	
Linseed oil	160	118	138
Corn oil	118	107	135
Olive oil	85	81	120
Lard	67	73	125
Crisco	69	70	126
Coconut oil	13	54	110

There is a distinct reversal in the average iodine number of the serum lipids. In the group fed on the low fat diet the total lipids of the serum had an average iodine number of 90, while in the cases of the animals reared on coconut oil the iodine number was 110. Thus it appears that a regimen low in fat depresses the degree of unsaturation of the serum lipids more than a diet fairly high in fat but containing mostly saturated fatty acids. This observation, coupled with that of the differences found between the olive oil and lard and Crisco reared animals, suggests the probability that the unsaturated acids are selectively retained by the blood. In general the results are considered to support the view that the rat depends upon the diet for its unsaturated fatty acid supply. The fact that the blood picture can be changed so readily by dietary

means indicates the importance of regulating this factor in metabolic studies which involve the serum lipids. Our findings show that the same general relationship exists between the dietary fats and blood lipids as was found by Anderson and Mendel ('28) to exist between dietary fats and body fats.

SUMMARY AND CONCLUSIONS

1. When rats were reared on diets containing fats of various degrees of unsaturation the iodine numbers of the total lipids of the serum tended to vary directly with the iodine number of the dietary fats.

2. The values for the total lipids were found to vary inversely with the degree of unsaturation of the serum lipids.

3. The results indicate the possibility of a selective retention of the unsaturated fatty acids of the blood.

4. These data indicate that the character of the diet must be taken into consideration in the interpretation of metabolic studies involving the serum lipids, particularly as regards the degree of unsaturation of the fatty acids.

5. The results show that a positive correlation exists between the degree of unsaturation of food fat, depot fat and the blood fat.

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BASAL METABOLISM OF OKLAHOMA MEN AND CHILDREN

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ONE FIGURE

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For several years it has been doubted whether the northern standards of basal metabolism (DuBois or Benedict) can be generally used satisfactorily, and experimental evidence has actually shown these values for women to be too high for use in the south. Hafkesbring and Borgstrom ('26) reported basal metabolic rates for normal women in New Orleans that were below the northern standards. Tilt ('30) from a study of Florida women reported similar results. Coons ('31) in this laboratory from a study of 101 Oklahoma college women reported an average basal metabolic rate of —13.2%. The question has arisen as to whether Oklahoma men and children show a correspondingly low basal metabolism. Therefore, in order to make the study complete, data were compiled on the basal metabolic rate of eighty-four children of both sexes and seventy-five men from this State.

PROCEDURE

The Benedict-Roth recording metabolism apparatus was used for all tests. The usual precaution in testing each time for leaks and often for efficiency of the soda lime for CO_2 and H_2O absorption was observed. All tests were run early in the morning, the subject being in the post absorptive state and having rested 30 minutes just previous to the determination.

BASAL METABOLISM OF OKLAHOMA MEN

The seventy-five men whose metabolic rates were studied were chosen indiscriminately from men enrolled in this college. They were all normal, healthy young men, many of whom were in athletic training, and varying in age from 17 to 30 years; the majority falling within the range from 18 to 24. The data so obtained are summarized in table 1.

The data from these subjects were assembled into age groups as indicated for convenience in handling and because the number of subjects of each age studied was not sufficiently large to merit separate consideration.

An inspection of table 1 indicates the lowered basal metabolic rate shown by Oklahoma men. The average rate for all

TABLE 1

AGE	NUMBER	AVERAGE WEIGHT (KG.)	AVERAGE SURFACE AREA (M ²)	AVERAGE TOTAL HEAT PRODUCTION (CAL./24 HOURS)	AVERAGE (CAL./M ² PER HOUR)	AVERAGE B.M.R. (%)
17-19	27	65.75	1.79	1762.9	41.03	— 4.42
20-24	39	72.14	1.91	1761.3	38.40	— 6.34
25-29	9	72.60	1.87	1696.8	37.80	— 6.20

age groups was found to be — 5.63%. Of all subjects studied 76.4% fell within $\pm 10\%$ deviation from the DuBois normal; 21.9% fell below — 10%; and 8.2% were below — 15%.

Figure 1 represents graphically the distribution of the basal metabolic rate results of all the subjects over the range from — 24% to + 18%. The fact that the mode is in the range from — 6% to — 12% would lead one to believe that the average basal metabolic rate of the entire group would be somewhere in that range rather than the calculated — 5.63%, but the close approximation of the 0 to — 6 frequency to that of the mode along with the shewness of the variation curve to the right accounts for this.

BASAL METABOLISM OF OKLAHOMA CHILDREN

The basal metabolic rates of eighty-four children (forty-two boys and forty-two girls) ranging in age from 5 to 15 years

were determined. These children might be referred to as a privileged group having been selected from homes where they had the advantages of a select diet and very favorable surroundings. The parents appeared to be normal, healthy individuals. In every case the child was brought from his bed following a night's sleep, and then allowed to rest for an additional 30 minutes. It should be noted that splendid co-operation was obtained from these children. The data so obtained should be indicative of the normal.

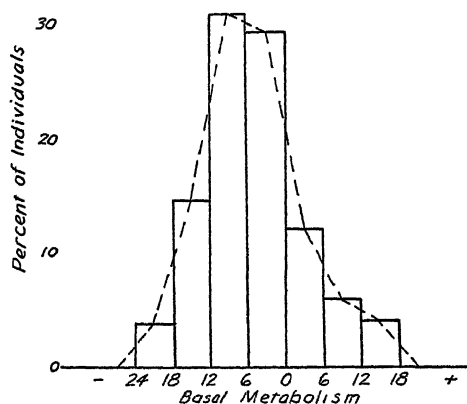


Fig.1 Frequency polygon and curve showing variation in basal metabolism of seventy-five Oklahoma men.

The results of this group of tests are summarized in two parts: table 2 for girls and table 3 for boys.

The material in the two preceding tables seems to indicate that the basal metabolic rate of the children falls lower from the normal as their age increases, this lowering being most marked with the age group of girls at puberty. The more striking lowering of the basal metabolic rate of young men and young women just beyond these ages is shown by the data of the older boys in the first part of this report. Unpublished data not here represented further substantiate this view for older girls.

The fact that the younger children do not show the low basal metabolic rate of the older ones and of men and women leads one to postulate that they are growing rapidly and since the basal metabolic rate is thought to be a function of the active protoplasmic tissue the rates of these children are as high as those of the north; the deviation from the northern standards coming with the approach of maturity.

TABLE 2

Girls

AGE	NUMBER	AVERAGE WEIGHT (KG.)	AVERAGE SURFACE AREA (M ²)	AVERAGE TOTAL HEAT PRODUCTION (CAL./24 HOURS)	AVERAGE (CAL./M ² PER HOUR)	AVERAGE B.M.R. (%)
5- 7	20	21.27	0.83	866.85	43.36	+3.36
8-11	16	30.57	1.08	1332.29	51.40	+2.63
12-15	6	42.37	1.37	1713.05	52.10	-6.59

TABLE 3

Boys

AGE	NUMBER	AVERAGE WEIGHT (KG.)	AVERAGE SURFACE AREA (M ²)	AVERAGE TOTAL HEAT PRODUCTION (CAL./24 HOURS)	AVERAGE (CAL./M ² PER HOUR)	AVERAGE B.M.R. (%)
4- 7	12	21.36	0.83	861.60	43.20	+1.72
8-11	24	31.81	1.12	1457.68	54.23	-2.34
12-14	6	41.86	1.36	2071.01	63.45	-1.12

CONCLUSIONS

1. The basal metabolism of Oklahoma men is lower than the DuBois standards, the average of seventy-five normal men being — 5.63% deviation from the DuBois normal.

2. The basal metabolism of Oklahoma children agrees with the northern standards while they are very young but gradually become lowered with increasing age as shown in these child studies and in the results of younger men here reported, and for girls previously reported from this laboratory.

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VITAMIN B₂ DEFICIENCIES AS AFFECTED BY DIETARY CARBOHYDRATE

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FIVE FIGURES

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In the course of certain attempts to produce and to prevent cataracts in rats by dietary means there were observed in this laboratory striking differences in the response of rats to basal diets similar in all respects except as to the carbohydrate constituent. Lactose was used in one group of experiments because of the now well-established cataractogenic effect of that sugar (Mitchell and Dodge, '34; Yudkin and Arnold, '35) and vitamin B₂ deficiencies were produced both with this and with the more usually employed cornstarch diet, pursuant to Day, Langston and O'Brien's ('31) finding that cataracts may occur in rats having such deficiencies. It was found at once that while cataracts are produced more or less regularly on lactose-containing diets, the other more usual B₂ deficiency symptoms could not be obtained. This led to the use of sucrose as well as of lactose and cornstarch with varying single and multiple B₂ deficiencies.

The whole problem of the nature, function and even the number of factors included under the designation vitamin B₂, as the British writers have employed the term, is at present too confused for any attempt at review here. Suffice it to say that we have been satisfied by the evidence brought forward by Lepkovsky, Jukes and Krause ('36), Elvehjem and Koehn ('35), Birch, György and Harris ('35), Gorter ('36), and others that at least three distinct factors are involved,

1) riboflavin, 2) an adsorbable heat stable probably basic substance called B_6 by György ('34), and factor 1 by Lepkovsky, Jukes and Krause ('36), 3) one or more unadsorbable factors called filtrate factor by Lepkovsky, Jukes and Krause ('36), B_2 by Elvehjem and Koehn ('35), antiblacktongue or P-P factor by Birch, György and Harris ('35). We have chosen to call these flavin, B_6 and filtrate factors respectively.

The plan involved the use of three similar basal diets with sucrose, lactose or cornstarch as carbohydrate constituent, adequate amounts of the fat-soluble vitamins fed as cod liver oil, crystalline vitamin B_1 (Merck) (10 micrograms per rat per day) as source of vitamin B_1 and one, two or all three of the B_2 factors. There were thus three groups of rats each on the following four regimes: without any of the B_2 factors; with only one, that is flavin or B_6 or filtrate factor; with each of the three possible combinations of two, that is flavin and B_6 , flavin and filtrate factor, filtrate factor and B_6 ; and finally with all three factors. Twenty-seven groups were used, each made up of four to ten animals. Several of the comparisons were repeated three to five times. The growth curves used in this report, however, were taken from concurrent groups carefully chosen from comparable litters since it has become clear that all feeding experiments of this kind must be judged by the parallel performance of littermate controls.

The diet used had the following composition: casein, purified by repeated extractions with hot 95% alcohol and with cold 60% alcohol, 22; Crisco, 9; Osborne and Mendel salt mixture, 4; carbohydrate, 65. In some of the lactose diets a lower proportion of casein was used, 18 or 15%, but no differences in the relative severity of the vitamin deficiencies resulted. The lactose was exposed to strong daylight in thin layers for 7 or 8 days before it was incorporated in the diet and exhibited no fluorescence under 'black light.'

There was little coprophagy among the rats fed these diets. Wide-mesh floor screens on individual cages were used and careful observation made to detect any feces consumption. A small number of suspected animals was removed from the groups.

In most cases the rats, taken at 21 to 28 days of age, were depleted by use of the B₂-free diet for 3 or 4 weeks at which time the weights were found to be stationary or declining except in the case of the lactose groups.

A single level of dosage was chosen for each of the three supplements and only one source of each supplement. Pure crystalline lactoflavin furnished by Dr. S. Lepkovsky and by Vitab Products, Inc. of San Francisco was used at the level of 20 micrograms daily. A series of assays of this material established its high potency, comparable with that reported by Copping ('36), by Ansbacher, Supplee and Bender ('36), and of course, by Lepkovsky and Jukes ('36).

The B₆ preparation was made from autolyzed wheat germ according to the suggestion of Birch and György ('36) and was fed in daily amount equivalent to 0.5 gm. of wheat germ. This extract was tested by Dr. T. H. Jukes on chicks and found to be free from the filtrate factor. Rat assay has confirmed this. The filtrate factor was a rice bran concentrate furnished by the kindness of Vitab Products, Inc. This product was practically free from flavin but contained appreciable amounts of B₆. In all the tests involving the filtrate factor therefore the presence of a small amount of B₆ must be postulated. The standard dose used was 0.5 cc. of the concentrate per rat per day.

The feeding experiments were carried on for varying periods from 12 to 36 weeks, but usually for 14 weeks. Growth, blood and urine sugar, blood and urine calcium, eye lens changes, skin and hair changes were recorded.

THE EFFECT OF LACTOSE

The outstanding findings in the groups on the lactose diet were the complete absence of dermatitis, frequent appearance of cataracts, and subnormal but persistent growth even on the entirely B₂-deficient diet (Morgan and Cook, '36). Addition of either flavin or B₆ or both had little or no effect in improving the growth of these animals. When the filtrate factor was given however either alone or with the other two

factors, a large increase in growth rate resulted and a definite decrease in severity and speed of appearance of the cataracts. With filtrate factor alone added (it being understood that B_1 is present in all cases) the growth achieved as seen in figure 1 is the same as that with 0.5 gm. brewers' yeast (Northwestern) daily. When the flavin and B_6 were also given, the growth is

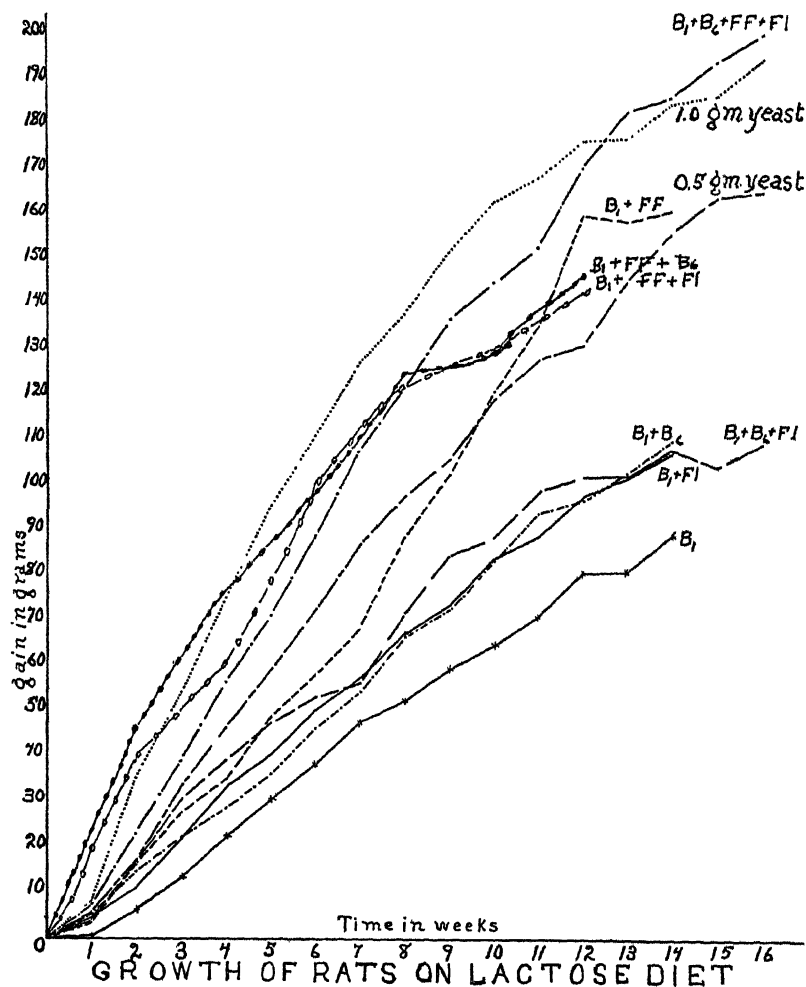


Fig. 1 Growth of young rats on lactose diet with one two, three or all four of the B vitamins: B_1 , flavin (FI), filtrate factors (FF), vitamin B_6 (B_6).

seen to be equal to that promoted by 1.0 gm. daily of the yeast. Apparently 0.5 gm. yeast supplies insufficient amounts of one of the B₂ accessories since 1.0 gm. of yeast is needed to equal the performance of the group given the four factors separately.

All of the lactose-fed rats had much enlarged ceca filled with yellowish white semi-solid contents, apparently mostly unabsorbed lactose. Early in the feeding period some showed diarrhea and a few died during these attacks. Usually after a week or two the diarrhea ceased and the animals appeared to be normal in all respects except for the cataracts and a sub-normal rate of growth.

In an earlier experiment groups of rats were fed a similar diet but with 50% lactose and 20% cornstarch instead of 70% lactose and with tiki-tiki as source of all the B vitamins. These had less mature and more delayed cataracts but considerably improved growth when compared with those on the 70% lactose diet. When the proportion of lactose was further reduced to 30%, the cataracts were fewer and less severe but growth was much decreased. These relations are shown in figure 2. Apparently the tiki-tiki was supplemented successfully by the vitamin-forming activity of the lactose at the 50% level, insufficiently at the 30% level and at the 70% level the toxic effect of the lactose became more pronounced than its favorable effect. With cornstarch alone the tiki-tiki allowed very little growth, no cataracts developed but severe dermatitis or hair loss usually occurred. It is probable that the tiki-tiki was lacking chiefly in flavin.

Since B₆ or flavin or both along with the 70% lactose diet brought about little improvement, it would appear that either the lactose contained considerable amounts of these two factors or that they were formed possibly by bacterial activity in the intestine from which adequate quantities were absorbed. Since the addition of the filtrate factor brought about obvious improvement, it must be assumed that the lactose carries none of this and that none is elaborated in the intestine.

In order to determine whether the intestinal contents contain the B₆ and flavin the contents of the ceca of sixty-five lactose-fed rats were washed out with 60% alcohol, filtered, the filtrate concentrated and fed to six rats depleted on the cornstarch diet. These animals resumed growth at a rate

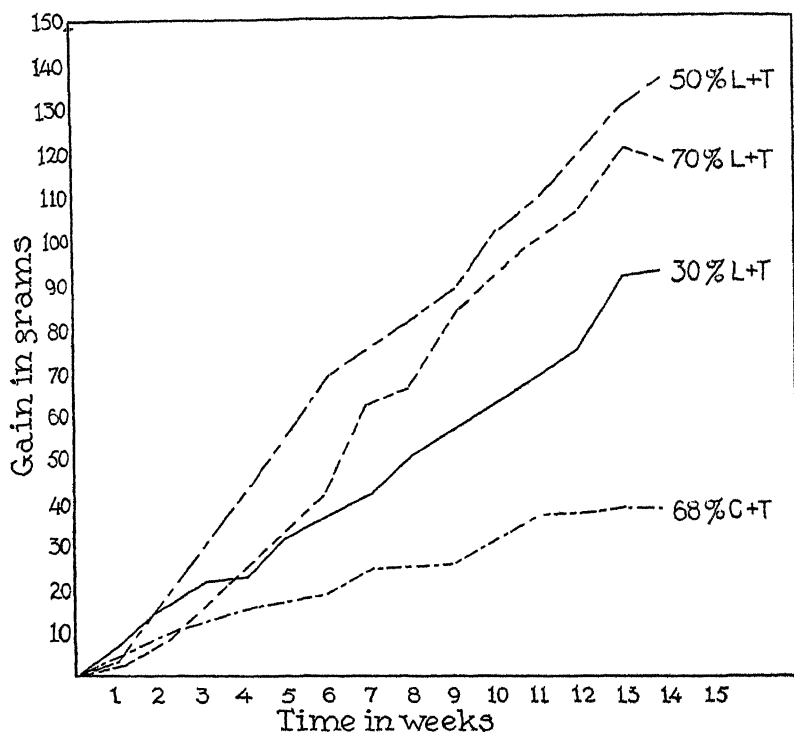


Fig. 2 Growth of young rats on diets containing 30, 50 and 70% lactose (and 40, 20 or 0% cornstarch) and 68% cornstarch with tiki-tiki (rice bran concentrate) as sole source of the B vitamins.

closely paralleling that of littermates on the lactose diet without supplements. The equivalent of the contents of one caecum per rat promoted growth for 3 or 4 days. When the caecum material was no longer given, the growth of these rats continued for 1 week then leveled off as in the negative cornstarch group.¹

¹ These observations were made by Dorothy V. Rundle.

THE EFFECT OF CORNSTARCH

The rats on the cornstarch diet grew very little without supplements, sometimes developed dermatitis but had no cataracts. See growth curves in figure 3. Their growth was improved by flavin but the dermatitis became more severe and

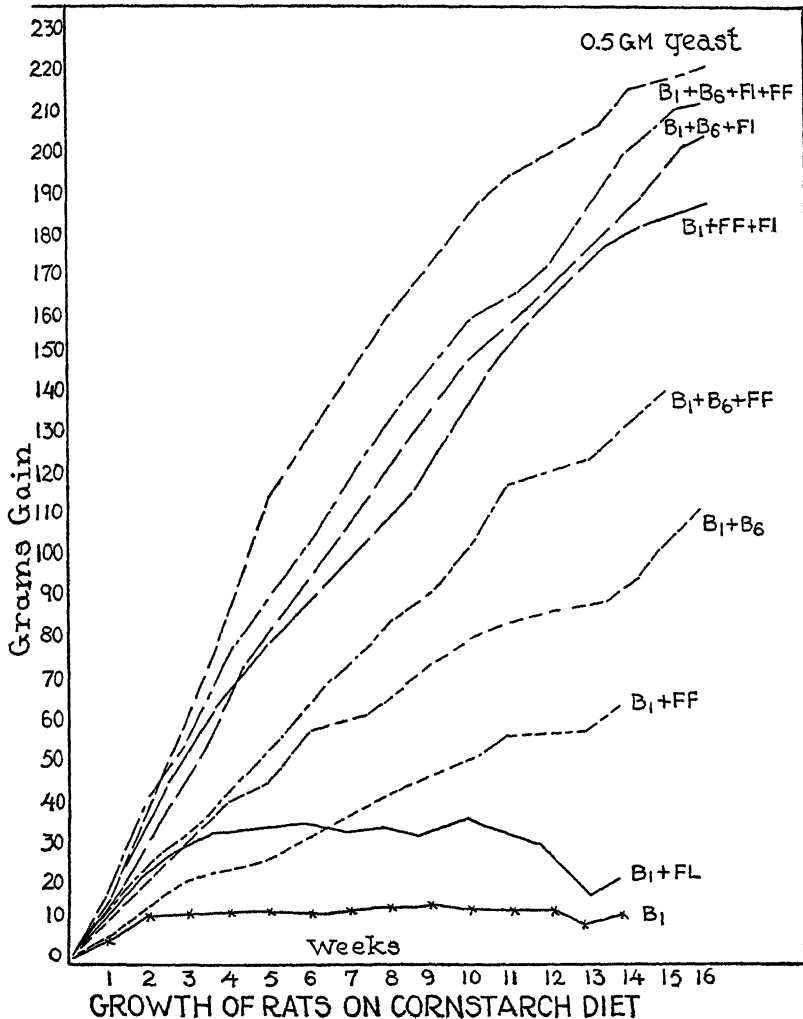


Fig. 3 Growth of young rats on cornstarch diet with one, two, three or all four of the B vitamins: B₁, flavin (FI), filtrate factors (FF), vitamin B₆ (B₆).

was of the usual 'specific' type with swollen red paws from which the skin was sometimes sloughed off, scabby denuded nose and forelegs and swollen encrusted ears. When B_6 was also given, the dermatitis did not appear and close to normal growth resulted. Addition of the filtrate factor merely increased the growth rate somewhat but alone produced little improvement.

The filtrate factor preparation which was used was assayed² for B_6 and flavin by being fed at two levels with flavin to one group of rats and with B_6 to another. No improvement was found in the latter by doubling or quadrupling the quantity of filtrate factor, which was taken to mean that the latter contained little if any flavin. Definite improvement in growth, sufficient to make these groups equal in performance to those receiving the standard dose of B_6 supplement, resulted however when the filtrate factor was doubled and quadrupled for the group given flavin but no B_6 . Apparently the filtrate factor in the quantity fed, 0.5 cc. per day, contained one-half the amount of B_6 in the daily dose of wheat germ preparation used as source of B_6 , that is one-half of the equivalent of 0.5 gm. wheat germ. It is not surprising therefore that when filtrate factor and flavin were given, fairly good growth resulted on all diets.

We were puzzled by the good growth resulting from the addition of flavin and B_6 to the cornstarch diet and by the relative ineffectiveness of additions of filtrate factor either alone or with the other supplements, as shown by the growth curves in figure 3. The supposition must be made either that the cornstarch carries appreciable amounts of filtrate factor or that it promotes intestinal formation of this factor. Since the B_6 preparation appeared to be free from filtrate factor by chick assay and by its effect with the sucrose and lactose diets, no other source of the filtrate factor than the starch can be found.

Hogan and Richardson ('34) have reported cure of florid dermatitis in rats fed a sucrose diet by concentrated hot

² These assays were made by Dorothy V. Rundle.

alcohol extracts of cornstarch, the condition cured being possibly due at least in part to filtrate factor deficiency. Certainly the factor which might be expected to adhere to cornstarch is not the filtrate factor which has begun to be identified through clinical cures of pellagra and blacktongue with the P-P of Goldberger. Several recent reports moreover, emphasize the relative poverty of cereals in general and maize in particular in all these factors except B₆.

Many of the studies with rats in which pellagra-like conditions were produced have been carried on with basal diets containing large amounts of cornstarch, rice starch or cereal mixtures. The work of Birch, György and Harris ('35) which led to their conclusion that rats do not need or can synthesize the third factor, that is additional to flavin and B₆, was done with a diet containing 68% cornstarch. If the filtrate factor is not actually carried by the starch usually employed, it may be that the bacterial flora favored by the presence of the starch in the rat's intestine synthesize it.

The obvious inconsistency of the presence of the filtrate factor in cornstarch along with the role of corn (maize) as a prominent part of pellagra-producing diets raises again the question of the specificity of the filtrate factor as the pellagra-preventing substance or at least of the singleness of character of this accessory or accessories.

THE EFFECT OF SUCROSE

The rats fed the sucrose diets supplemented with B₁ only developed dermatitis regularly if they survived long enough, grew not at all and usually died in a few weeks. Some growth resulted when any one of the three factors was given, the filtrate factor probably because of its B₆ contamination being most effective (fig. 4). When any two of the factors were given together, improvement over that seen on any one alone resulted, and when all three were given, normal animals were produced nearly as large as those given 0.5 gm. yeast daily. The improved growth obtained by the addition of each factor is in almost mathematical relation to the growths obtained

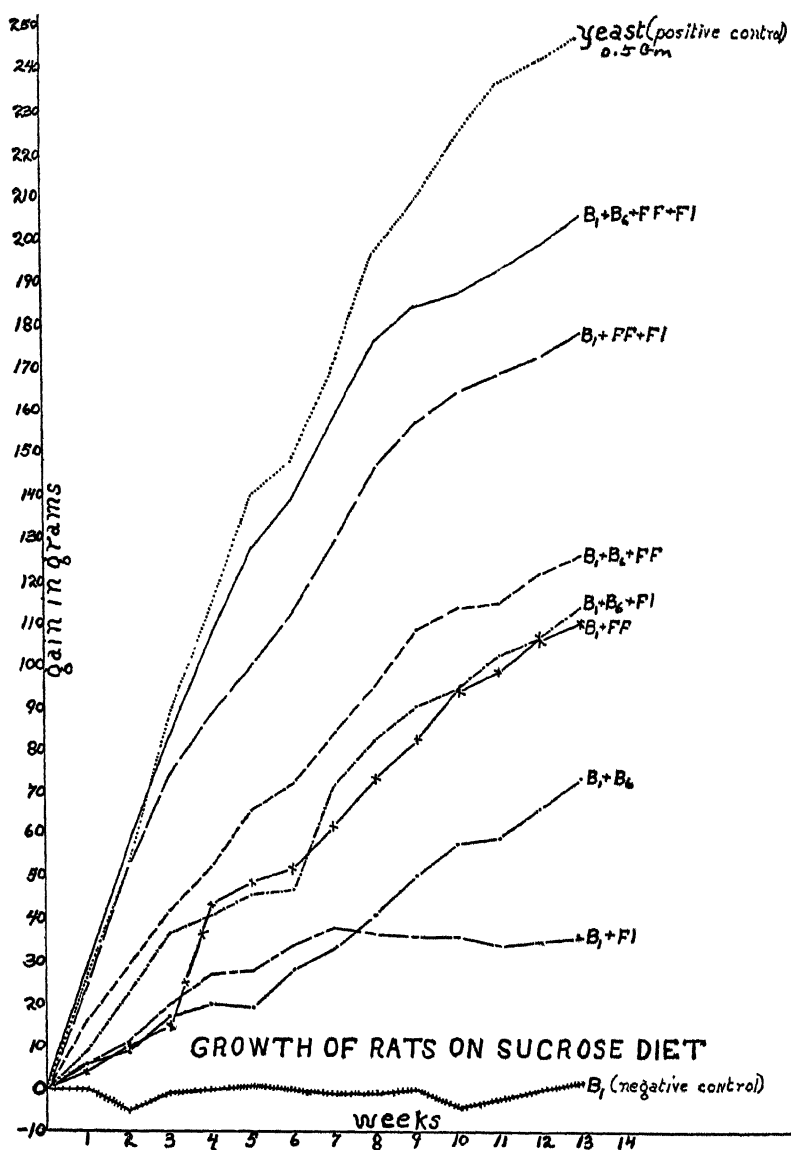


Fig. 4 Growth of young rats on sucrose diet with one, two, three or all four of the B vitamins: B₁, flavin (FI), filtrate factors (FF), vitamin B₆ (B₆).

when one or two are progressively missing. Thus in 14 weeks flavin alone promoted an average gain of 35 gm., B₆ alone, a gain of 72 gm., filtrate factor alone, a gain of 108 gm. In the latter case it must be remembered that about one-half the standard dose of B₆ was present in the filtrate factor supplement. When flavin and B₆ were given together, the gain was 112 gm., almost an exact addition of the separate gains. When flavin and filtrate factor were given together, the gain was 177 gm., close to the normal obtained with flavin, B₆ and filtrate factor, 204 gm. When B₆ and filtrate factor were given together, the gain was 125 gm., 73 of this ascribable to the B₆. Apparently the sucrose diet is completely free from all of these factors and also produces none of them in the intestine. It would seem therefore to be the carbohydrate of choice for basal diets for rats in the study of the vitamin B₂ complex. This conclusion was reached also by Halliday and Evans ('37) who found the depletion period shorter and deficiency symptoms more severe with sucrose instead of cornstarch diets.

The studies of Guerrant, Dutcher and Tomey ('35) with diets containing various carbohydrates and deficient in all the B vitamins have indicated the synthetic activities of the flora promoted by dextrin and to a slighter extent, lactose. The overwhelming effect of vitamin B₁ deficiency apparently obscured the effect upon the B₂ factors in this case. The recent work of Bender, Ansbacher, Flanigan and Supplee ('36) has differentiated the effect of dextrin however since B₁ and lactoflavin were supplied. No dermatitis occurred on the dextrin ration but with a similar sucrose diet dermatitis was quite severe. The addition of rice polish concentrate cured the dermatitis in the latter animals and produced practically normal growth. But a flavin-free milk concentrate had no such effect. Whether the addition of the milk concentrate improved the growth of the dextrin-fed rats is not evident and there is no statement as to the use of the rice polish concentrate with the dextrin ration. It is impossible to judge therefore whether the dextrin provides both B₆ and filtrate factor in adequate amounts. Obviously it does not provide

flavin as does the lactose. There is apparently some difference between the intestinal activities or the original contaminations of these two carbohydrates. It seems clear however from the absence of dermatitis in both lactose and dextrin-fed rats that B₆ is produced in both cases.

BLOOD AND URINE ANALYSES

As determined by the method of Shaffer and Somogyi ('33) the blood sugar of the rats on the sucrose diet was within the usual normal range, 97 to 121 mg. per 100 cc., in all cases both with and without deficiencies. The same was true of those on the cornstarch diet in which the range was 108 to 125 mg. per 100 cc. Those on the lactose diet however had blood reducing sugar values of 158 to 241 mg. per 100 cc. Such high blood sugar values are in agreement with the findings of Day ('36) and of Mitchell, Merriam and Cook ('37). No attempt was made to differentiate glucose from galactose in the blood of the lactose-fed rats. A few animals on lactose plus vitamin B₁ were given small frequent doses of insulin zinc for 3 or 4 weeks in order to determine whether the cataracts produced by such a diet might be thus averted. No change in severity or early appearance of the cataracts resulted but the reducing sugar in the blood of these animals was found to be lowered to the same extent as in cornstarch-fed animals. This observation is contrary to the report of Mitchell, Merriam and Cook ('37), who found blood galactose, assuming that most of the hyperglycemia was due to galactose, unaffected by insulin.

Urine sugars as determined in samples of undiluted rat urine collected with special precautions to avoid contamination were found to be lowest on the starch diet, 44 to 117 mg. per 100 cc. of urine, intermediate on the sucrose diet, 159 to 480 mg. per 100 cc. of urine and highest on the lactose diet, 254 to 956 mg. per 100 cc. of urine. With sucrose the larger urine sugar output was seen on the deficient diets.

Urine calcium was determined also on the undiluted urine and was found to be largest on lactose diet, 59 to 171 mg.

per 100 cc. of urine. On the starch and sucrose diets the range of excretion was the same, 3 to 27 mg. per 100 cc. of urine. This effect of the lactose is perhaps only the result of the increased calcium absorption first reported by Bergeim ('26) as resulting from the presence of lactose in the intestine.

THE GRAYING OF RAT HAIR

An interesting symptom which developed in many of the filtrate factor-deficient animals was the graying of hair in regular patterns. This was most evident in the black rats but could be seen in gray and hooded animals as well. In very few cases was the graying evident in those which received only vitamin B₁. The rats which grew well and were relatively free from dermatitis or alopecia in nearly all cases became gray. These were chiefly the rats on sucrose diet which received no filtrate factor. Those on cornstarch in many cases grayed also but only after a prolonged period. The phenomenon was first observed in animals given the starch diet and small amounts of wheat germ, and all of these were cured of the depigmentation by the filtrate factor preparation from rice bran. In all cases when B₆ was present in the diet as well as flavin the graying was most severe.

Three black rats of the same litter fed the sucrose diet are shown in figure 5. Rat no. 10 was given all three factors throughout the experiment which continued for 24 weeks. This animal was normal in all respects at all times and continued to have a glossy black coat. Rat no. 11 was given the B₆ preparation only for the first 12 weeks during which time its fur became streaked with gray. The filtrate factor preparation was then added in standard amount to the diet of no. 11. After 8 weeks the fur of this animal had again become glossy and black. Rat no. 12 was given B₆ and flavin only for these 20 weeks with increasing graying of the fur, bloody ears and nose and emaciation. During the last 8 weeks 0.17 mg. of copper as copper sulfate was given rat no. 12 daily. The photograph shown in figure 5 was taken after the experiment had progressed for 20 weeks. Obviously the two rats

given the filtrate factor have normal fur while the rat lacking the filtrate factor is in poor condition with completely depigmented fur. The copper had no curative effect on the depigmentation. Gorter ('35) has recently claimed that the depigmentation is due to lack of copper and that it may be prevented or cured by the administration of minute amounts of copper

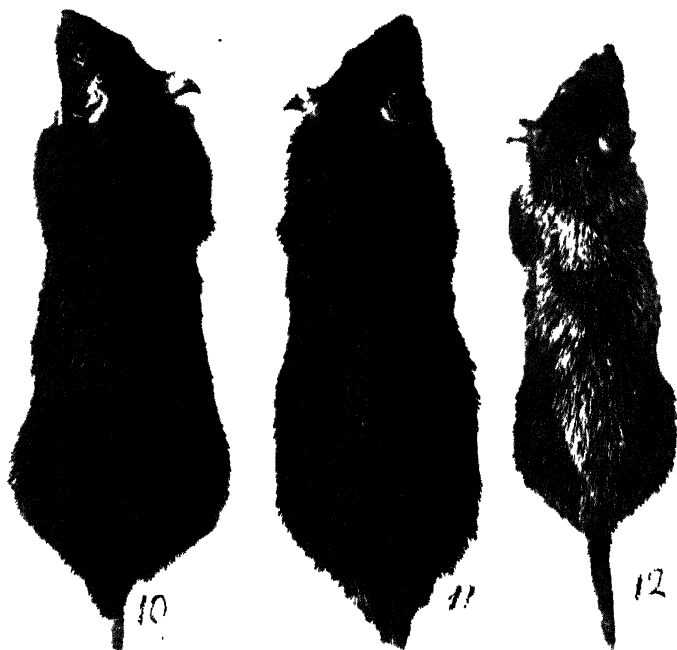


Fig. 5 Littermate black rats on the sucrose diet. No. 10 was given all of the B_2 vitamins throughout the experiment which continued for 24 weeks. No. 11 was given B_2 only for 12 weeks, then filtrate factor. No. 12 was given B_2 and flavin (no filtrate factor) but was given additional Cu latter part of experiment.

salts. The specificity of copper may be questioned in these cases since all rats in this laboratory obtain their water from bottles equipped with pure copper drinking tips. After the photograph shown in figure 5 was taken, rat no. 12 was given the filtrate factor in double the standard daily dose for 4 weeks at which time its fur had become black again, it had increased in size and had become normal in all respects.

Another group of some forty young black rats have been placed on the sucrose diet with vitamins B₁ and B₆ and flavin but without filtrate factor. Nearly all of these animals have shown marked graying of the fur within 8 to 12 weeks and rapid resumption of the normal color when filtrate factor preparations made from rice bran and from liver were fed. Control animals given the filtrate factor from the beginning have retained normal fur color. We conclude that the filtrate factor or factors prevents and cures graying of rat hair.

SUMMARY

When lactose in sufficient amount is incorporated in the basal diet, of the three necessary factors now recognized in the vitamin B₂ complex, both flavin and vitamin B₆ need not be given separately and are apparently elaborated in the intestine of the animals. The filtrate factor however must be given in order to obtain normal growth. In no case did dermatitis develop in lactose-fed rats even when the proportion of lactose was lowered to 30%, but a large proportion of the rats developed cataracts. The severity of the cataract occurrence was mitigated when all the vitamin B factors were supplied, and this prophylactic effect seemed to inhere in the filtrate factor particularly.

When cornstarch is used as the carbohydrate of the basal diet, dermatitis and relative failure of growth result unless B₆ and flavin are supplied. The filtrate factor appears to be present in limited amount in the starch or to be produced in the intestine when cornstarch is used. No cataracts occurred on any of the starch or sucrose diets.

When sucrose is used as the carbohydrate of the basal diet, earlier and more severe dermatitis and growth failure occur unless all three of the factors are supplied. Better growth response was obtained with sucrose than with starch when one or more of the factors was supplied and better response with starch than with lactose.

Graying of the hair of black rats was seen on most of the diets which lacked the filtrate factor and this was prevented or

cured by administration of the rice bran concentrate which was used as source of the filtrate factor.

Blood and urine reducing sugar of the rats on lactose were much increased over that of the starch-fed group. Urine calcium was also much increased in this group. The sucrose diets produced normal blood sugar values but when one or more of the B₂ factors was lacking, high urine sugars. Some clue to the mode of action of these factors may be found in this behavior.

CONCLUSIONS

Lactose favors the production in the intestine of rats, probably by microorganisms, of both flavin and vitamin B₆ but not the filtrate factor.

Cornstarch either carries with it or favors the production of the filtrate factor only.

Sucrose neither carries nor produces any of the vitamin B₂ factors and is therefore the carbohydrate of choice for the study of these factors.

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ETIOLOGY OF SEBACEOUS GLAND ATROPHY IN THE RAT IN AVITAMINOSIS ¹

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EIGHT FIGURES

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INTRODUCTION

Albino rats on vitamin G ² deficient diets regularly show atrophy of the sebaceous glands and thinning of the epithelium as demonstrated by microscopic sections of the tail (Smith, '32). These findings have been confirmed and extended by Smith and Sprunt ('35) who showed that the lesions were independent of the initial age of the rat or the season of the year. The changes in the sebaceous glands and epithelium of the tail were demonstrated in the living animals by biopsy. Autoclaved yeast was uniformly effective both in preventing and in curing this symptom complex.

The same type of epithelial and sebaceous gland lesion occurred also in rats on vitamin A and vitamin B deficient diets, but was less severe. The lesions did not occur on Steenbock and Black's rickets-producing diet no. 2965. It has been suggested by Smith and Sprunt ('35) that the changes noted in rats on vitamin A and B deficient diets might be due to a secondary shortage of vitamin G precipitated by insufficient intake of food or faulty absorption of this factor from a basic diet. The present study was designed to test this assumption.

¹ Part of the expenses of this work was covered by grants from the Rockefeller Foundation, New York, N. Y., and the Lederle Laboratories, Pearl River, N. Y.

² Vitamin G as used in this report refers to all the factors of the complex occurring in autoclaved yeast.

EXPERIMENTAL

Animals. Young albino rats of the Wistar strain were used. The ages ranged from 27 to 33 days. They were housed in experimental cages with raised screen bottoms.

Grouping. Two groups of rats were used. Group I, consisting of forty-nine rats, served for the vitamin A study and group II, consisting of 135 rats, served for the vitamin G study. Each group was subdivided into three groups a, b and c, for the purpose of contrasting the two deficiencies under slight dietary variations.

1. Group I-a (seventeen rats) was placed on a basal diet deficient in vitamin A, to which vitamin G was added. Group II-a (thirty-five rats) was placed on a basal diet deficient in vitamin G to which vitamin A was added. This was the type of diet used in our previous studies. Under the influence of the original deficiency the animal's food intake decreases, so that one or more secondary deficiencies might be expected. To test this possibility, the next variation was made.

2. Groups I-b (twenty-one rats) and II-b (fifty-five rats) were placed on vitamin A and vitamin G deficient diets exactly as were the rats in groups I-a and II-a. In this experiment, however, the vitamin G of the A-deficient diet and the vitamin A of the G-deficient diet were fed individually, not mixed with the basal diet. The level of intake of these supplementary vitamins was thus maintained even after food consumption began to decrease.

3. Groups I-c (eleven rats) and II-c (forty-five rats) were positive control groups receiving diets identical with those of groups I-a and II-a except that group I-c received in addition a source of vitamin A and group II-c a source of vitamin G.

Diets. The A-free diet, A-a,³ fed to group I-a, consisted of casein (hot alcohol extracted) 18%, Osborn and Mendel salt mixture 4%, dry brewers yeast 10%, cornstarch 67%, sodium chloride 1%, and 6 drops of viosterol (250-D) per kilo of diet.

³ The large letter indicates the vitamin deficiency and the small one the group receiving it.

The diet A-b, fed to group I-b, had approximately the same ingredients except that crystalline vitamin B⁴ and autoclaved yeast replaced the untreated yeast. This was done to maintain the level of vitamin G as autoclaved yeast. The minerals 4%, casein 20% and starch 76% were mixed in the basal diet and the yeast and vitamin B were fed separately.

TABLE 1
Diets

INGREDIENTS	A-a	A-b	A-c	G-a	G-b	G-c
	%	%	%	%	%	%
Casein hot. alc. extd.	18	18	18			
Casein alc. extd.				18	18	18
Cornstarch	67	78	67			
Osborn and Mendel salts	4	4	4	4	4	4
Brewers yeast	10		10			
Irradiated ergosterol (250-D)	0.5 gm. per kilo of diet					
Sodium chloride	1		1			
Sucrose				68	70	68
Butter fat				8	8	8
Cod liver oil				2		2
Daily individual feeding						
Crystalline vitamin B		20 γ		20 γ	20 γ	20 γ
Autoclaved yeast		0.5 gm.				0.5 gm.
Cod liver oil			2 drops		2 drops	
Irradiated ergosterol		1 drop (w) ¹				
Lactoflavin				20 γ	20 γ	20 γ

¹ w = weekly.

Diet A-c, fed to group I-c, was identical with diet A-a except that each rat in this group received in addition 2 drops of cod liver oil daily.

The G-deficient diet G-a, fed to group II-a, consisted of casein (alcohol extracted) 18%, Osborn and Mendel salt mixture 4%, butter fat 8%, cod liver oil 2%, sucrose 68%, mixed into the basal diet and supplemented with pure crystalline vitamin B and purified lactoflavin⁵ fed individually to each rat.

⁴ Furnished by the Merck Chemical Co.

⁵ Furnished by the Winthrop Chemical Co.

The G-deficient diet G-b, fed to group II-b, was identical with diet G-a except that the cod liver oil was fed individually to each rat in order to maintain the level of vitamin A.

Diet G-c, fed to group II-c, was identical with diet G-a except that autoclaved yeast was fed in addition (table 1).

Method of giving supplementary feedings

Cod liver oil. Two drops daily of a good grade of oil was fed through a medicine dropper.

Autoclaved yeast. Brewers yeast was autoclaved $2\frac{1}{2}$ hours at 15 pounds pressure and 0.5 gm. of the dry product was placed in a small dish and fed daily.

Vitamin B. Ten milligrams of the natural crystals were dissolved in 25 cc. of distilled water and 1 drop of this solution, equivalent to 20 γ of the solid product, was fed daily to each rat.

Lactoflavin. One drop, equivalent to 20 γ pure lactoflavin, was fed daily to each animal.

Vitamin D. One drop per rat per week of viosterol (250-D) was given.

At the end of the experiment, or at death of any individual, cross sections of the tail were made at the base, mid-portion and tip. The details of the method have been published in a previous communication (Smith and Sprunt, '35).

Fig. 1 Photomicrograph of cross section of tail of rat in group I-a. Note thinning of epithelium and atrophy of the sebaceous glands. $\times 150$.

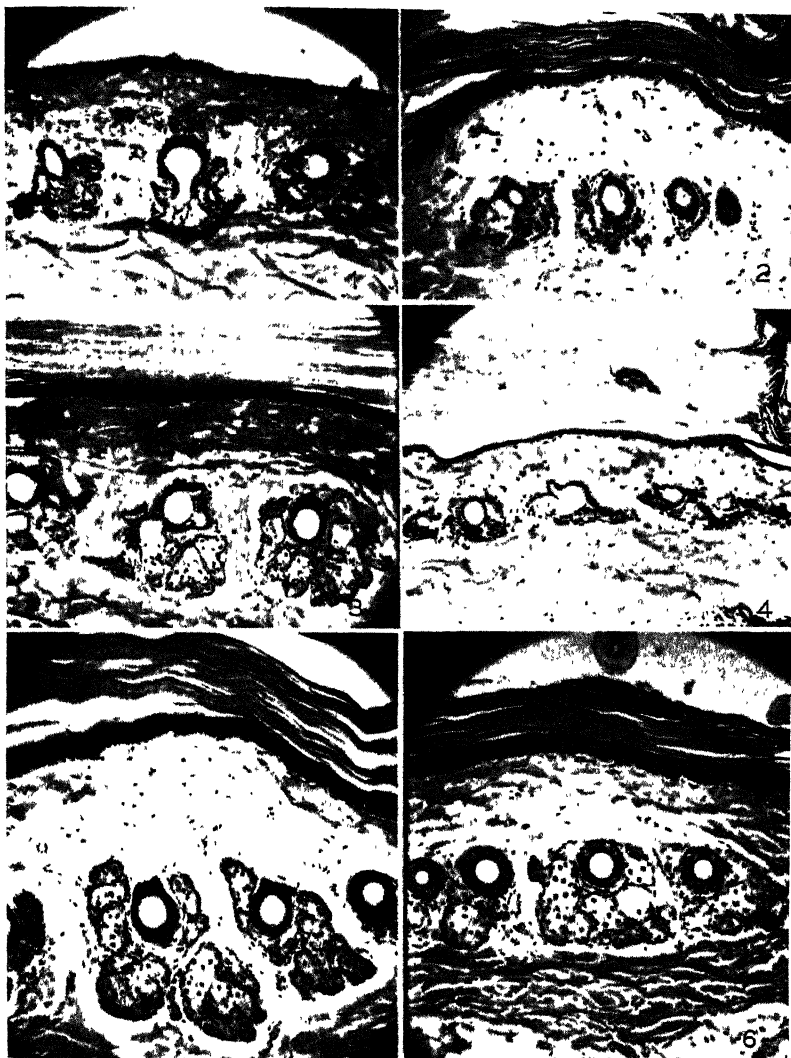
Fig. 2 Photomicrograph of cross section of tail of rat in group II-a. Note thin epithelium and almost completely atrophied sebaceous glands. $\times 150$.

Fig. 3 Photomicrograph of cross section of tail of rat in group I-b. These sebaceous glands are almost normal. $\times 150$.

Fig. 4 Photomicrograph of cross section of tail of rat in group II-b. There is marked thinning of the epithelium and almost complete atrophy of sebaceous glands. $\times 150$.

Fig. 5 Photomicrograph of cross section of tail of rat in group I-c. Positive control. Epithelium and sebaceous glands are normal. $\times 150$.

Fig. 6 Photomicrograph of cross section of tail of rat in group II-c. Positive control. Both epithelium and sebaceous glands are normal. $\times 150$.



Figures 1 to 6, microscopic sections of rat tails. 1, rat on A free diet with G mixed into basal; 2, rat on G free diet with A mixed into basal; 3, rat on A free diet with G fed separately; 4, rat on G free diet with A fed separately; 5, rat on A free diet + A (positive control); 6, rat on G free diet + G (positive control).

RESULTS

Group I. Vitamin A study

1. The rats on diet A-a grew at a practically normal rate for 9 weeks, maintained a plateau for 2 weeks and then the weight declined rapidly until death (fig. 7). Coincident with

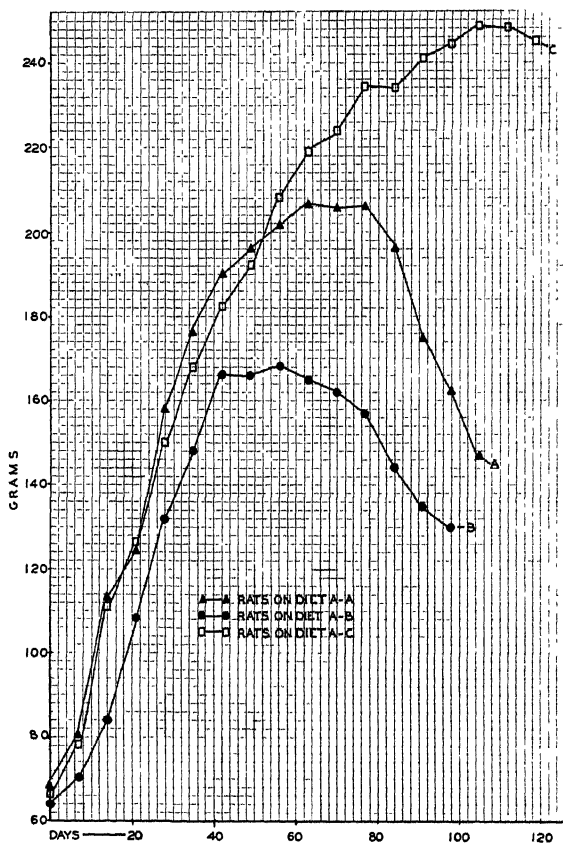


Fig. 7 Growth curves of rats in group I.

the decline in weight, symptoms of A deficiency developed, namely, somewhat roughened coat, xerophthalmia, and an unsteady gait. At autopsy fourteen out of seventeen rats had a large purulent abscess at the base of the tongue. The microscopic sections of the tail made at this time showed some atrophy and slight thinning of the epithelium (fig. 1).

2. The rats on diet A-b grew at a rate slightly less than normal for the first 6 weeks, showed little or no gain for 2 weeks followed by a gradual loss of weight until death. The development of eye symptoms, rough coat and unsteady gait, characteristic of vitamin A deficiency, coincided with the failure of growth. At autopsy thirteen of twenty-one rats had a large purulent abscess at the base of the tongue. The

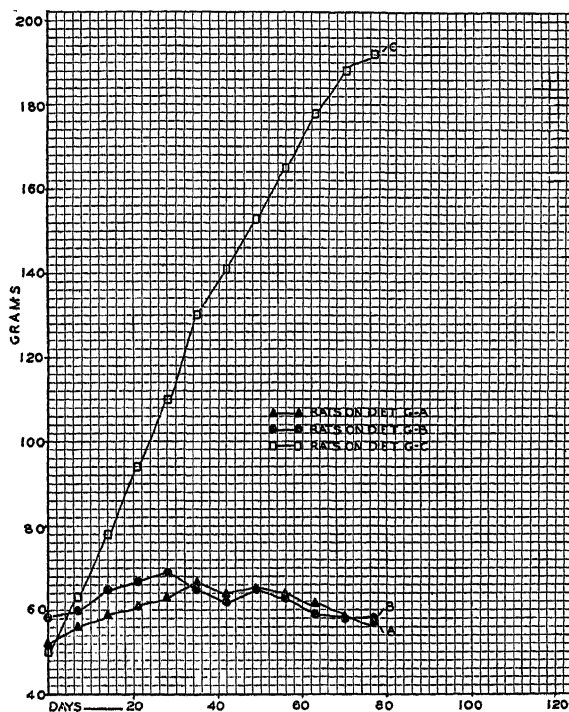


Fig. 8 Growth curve of rats in group II.

microscopic sections of the tail showed almost normal sebaceous glands and epithelium (fig. 3), evidence, we think, of the protective effect of vitamin G, as supplied by the autoclaved yeast.

It will be noted that the rats on diet A-a grew consistently better than the rats on diet A-b. This can be accounted for probably on the assumption that the autoclaved yeast and

crystalline vitamin B as fed to the animals do not provide exactly the same vitamin B and G factors that are found in untreated yeast. However, the factor missing or deficient is not significant in the present study, since the rats in group b, though growing at a slower rate, had the more normal sebaceous glands.

3. The rats on diet A-c grew normally (fig. 7) and remained symptom-free throughout the experiment.

Microscopic sections of the tail revealed a normal histology (fig. 5).

TABLE 2
Sebaceous gland lesions

GROUP	DIET	TOTAL NUMBER OF RATS	EXTENT OF SEBACEOUS GLAND LESION			
			Normal	+ ¹	++ ²	+++ ³
I-a	A-a	17	0	7	10	0
I-b	A-b	21	14	5	1	1
I-c	A-c	11	11	0	0	0
II-a	G-a	35	0	1	14	20
II-b	G-b	55	0	3	11	41
II-c	G-c	45	43	0	2	0

¹ Slight lesion.

² Moderate lesion.

³ Severe lesion.

Group II. Vitamin G study

1. The rats on diet G-a grew very poorly from the beginning and after 5 weeks lost weight gradually until the end of the experiment (fig. 8). Dermatitis, affecting the nose, mouth and all four paws, occurred regularly. Priapism was observed in the males and a failure to open the vaginal orifice in fifteen of eighteen females. Sections of the tail showed marked atrophy of the sebaceous glands and thinning of the epithelium (fig. 2).

2. The rats on diet G-b also grew very poorly (fig. 8) and exhibited the same symptoms as the rats on diet G-a. Sections of the tail showed marked atrophy of the sebaceous glands and thinning of the epithelium (fig. 4), thus indicating that no protection was afforded by feeding individually vitamin A as cod liver oil.

3. Rats consuming diet G-c grew normally (fig. 8) and appeared healthy. They exhibited no gross symptoms. Most of the females were pregnant at the end of the experiment. The tail sections revealed well-developed sebaceous glands and normal epithelium (fig. 6). (See table 2 for comparative histological findings.)

Vitamin B study

We could not contrast directly the effect of vitamin B with that of vitamin G, as we did in the case of vitamin A, owing to the early onset of anorexia, which interfered with the consumption of the autoclaved yeast. However, in a group of sixty rats fed crystalline vitamin B individually, while consuming a vitamin G deficient diet, there was no evidence of protection against the development of epithelial and sebaceous gland lesions.

DISCUSSION

Wolbach and Howe ('25 a and b) found slight atrophy of the sebaceous glands in rats on an A-deficient diet. As in group I-a, their dietary ingredients were all mixed into the basal diet. Our results with this type of diet correspond very closely to theirs. In our studies, however, when vitamin G in the form of autoclaved yeast was fed separately in group I-b, there was marked protection of the sebaceous glands. On the other hand, the feeding of vitamin A separately to rats in group II-b, on a G-deficient diet, failed to protect the sebaceous glands.

Much has been written about alterations in the sebaceous glands of human skin. These changes have been variously termed seborrheic dermatitis, phrynoderma, toad skin, permanent goose skin, etc. Such lesions have been observed in association with pellagra (Bigland, '20; Biggam and Ghalioungui, '33; Corkill, '34; Stannus, '12; Castellani and Chalmers, '19; Smith and Sprunt, '35). Castellani and Chalmers describe seborrheic changes in pellagra as a "frequency of nasal or facial seborrhoea, which is to be especially

noted in the nose where the sebaceous follicles are very prominent and filled with plugs of sebaceous material."

Similar lesions occur also in association with keratomalacia (Pillat, '29; Frazier and Hu, '31; Nicholls, '31; Loewenthal, '33; Mackay, '34; Goodwin, '34; Aykroyd and Krishmann, '37; Eddy and Dalldorf, '37; Rao, '37). Rao in his excellent report gives a very good clinical and histo-pathological description of these skin changes which he attributed to a nutritional deficiency in which vitamin A is an important factor. The sites affected were mainly "the extensor surfaces of the arms, thighs and upper part (postero-lateral aspect) of the forearms near the elbow. Histopathologically the condition was characterized by a superficial non-inflammatory hyperkeratosis of the epithelium of the epidermis and hair follicles, distension of the mouths of the pilo-sebaceous follicles by horny plugs, atrophy of the sebaceous glands and impaired function of the sweat glands." Rao stated that the atrophy of the sebaceous glands appeared to be secondary to the hyperkeratinization of the epidermis and hair follicles.

In the present study atrophy of the sebaceous glands of rats resulted from a deficiency of vitamin G either as a primary deficiency or as a secondary deficiency conditioned by an inadequate supply of vitamin A.

SUMMARY

A study has been made of the part played by a deficiency of vitamins A and G as well as vitamin B in producing skin changes involving thinning of the epithelium and atrophy of the sebaceous glands in rats.

1. The skin alterations described above, and demonstrated by microscopic section of the tail, occur regularly in rats on a diet deficient in vitamin G.

2. Similar but less marked changes occur on vitamin A and vitamin B deficient diets if the source of G is mixed into the diet.

3. The sebaceous glands are almost normal, being only slightly altered, on a vitamin A deficient diet if the level of vitamin G is maintained by feeding the autoclaved yeast separately.

4. The sebaceous glands are not protected, however, on a vitamin G-deficient diet when the level of vitamin A is maintained by feeding cod liver oil separately.

5. The sebaceous glands are not protected on a vitamin G deficient diet when the level of vitamin B is maintained by feeding the crystalline material separately.

CONCLUSION

Atrophy of the sebaceous glands in the rat's tail is apparently due to a deficiency of some factor of the vitamin G complex.

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RIBOFLAVIN AND A FURTHER GROWTH ESSENTIAL IN THE TISSUES

QUANTITATIVE DISTRIBUTION AND THE INFLUENCE OF THE FOOD

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TWO FIGURES

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Without asking space to review the literature of the distribution in the tissues of the nutritionally-undifferentiated group of substances which give flavin reactions in vitro, we would here record briefly the results of feeding experiments made to ascertain 1) the quantitative distribution of riboflavin (lactoflavin, 'vitamin G') in the tissues of favorably nourished animals, and 2) the influence of the level of intake of riboflavin and a further growth essential upon the concentrations of these substances in the tissues. In the interest of brevity and clarity, we first present the experimental findings, and then discuss such previous work as bears upon the phenomena with which we are here concerned.

EXPERIMENTAL

The general plan of these experiments was similar to that followed in the previous work of this laboratory upon the distribution of vitamin A in the tissues (Sherman and Boynton, '25). Healthy young adult rats of known nutritional history were slaughtered, dissected at once, and weighed amounts of different tissues fed as sole source of riboflavin to test animals. For the quantitative measurement of the relative amounts of riboflavin thus found, the method described by Bourquin and Sherman ('31) was used, other

work in this laboratory having shown that riboflavin (lactoflavin) is the growth limiting factor under the conditions of this method (Booher, '33, '34; Bisbey and Sherman, '35) and that the 'vitamin G values' as measured in this laboratory are measures of riboflavin.

The animals whose tissues were under investigation were chloroformed and bled from the heart to obtain samples of blood for feeding and to free the tissues from surplus blood. Skeletal muscle was quickly removed, organs were dissected out with the minimum of connecting blood vessels and any adhering drops of blood were removed by absorption with filter paper; then weighed portions of the organs and tissues were fed immediately to preclude deterioration of riboflavin value. The material thus fed was always readily and completely consumed by the test animals. Tissue material was thus obtained and fed thrice weekly; but for convenience of comparison and interpretation the data are given on the usual basis of 'daily except Sunday' allowance, i.e., 'per day' meaning one-sixth of the actual amount consumed in the course of each week.

Figure 1 shows the average weight curves resulting from the feeding of the skeletal muscle, heart muscle, kidney and liver of rats which had been reared on our diet B or diet 13, a mixture of two-thirds ground whole wheat and one-third dried whole milk with table salt and distilled water. This food mixture contains about 6 γ of riboflavin per gram. Each curve represents the average record of from three to fourteen test animals. Blood, brain and spleen were fed to smaller numbers. The findings are therefore more quantitative for the four first-named, than for the three last-named, tissues. Curves 1 to 4 of figure 1 indicate about equal amounts of riboflavin in 1.5 gm. skeletal muscle, 0.3 gm. heart muscle, or 0.15 gm. of kidney or of liver. But in a comparison at a lower level of feeding (curves 5 and 6 of fig. 1) it appears that distinctly more riboflavin was contained in 0.075 gm. liver than in 1.0 gm. skeletal muscle. Thus these data indicate that the concentration of riboflavin was about five times

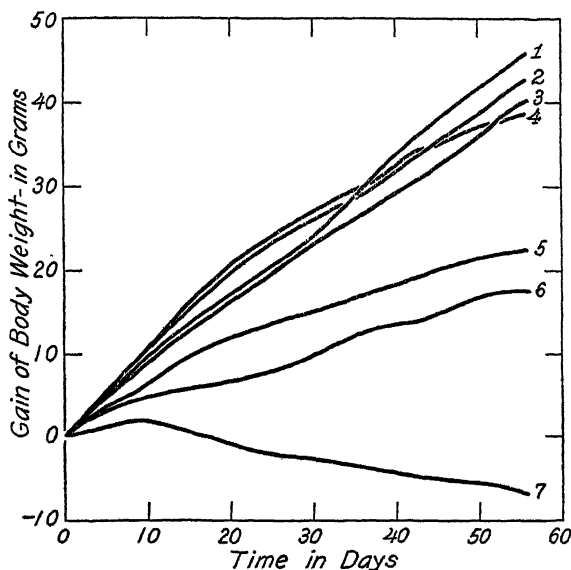


Fig. 1 Growth curves measuring distribution of riboflavin in the tissues of diet B rats: 1) From the feeding of 0.3 gm. per day of heart muscle; 2) of 0.15 gm. liver; 3) of 0.15 gm. kidney; 4) of 1.5 gm. muscle; 5) of 0.075 gm. liver; 6) of 1.0 gm. muscle; 7) negative controls.

as high in heart muscle, about ten times as high in kidney, and ten to twenty times as high in liver, as in skeletal muscle. Weight for weight, brain and spleen appeared to contain about two to three times as much, and blood only one-third as much, as skeletal muscle.

The basal diet which was fed the test animals in these measurements of the riboflavin values of tissues was the diet 555 of the accompanying table 1.

TABLE 1
Composition of diets 429 and 555

	Diet 429	Diet 555
Casein (extracted with 60% alcohol)	9	18
Osborne-Mendel salt mixture	2	4
Butterfat	9	8
Cod liver oil	1	2
Skim milk powder	30	..
Whole wheat (ground)	40	..
Cornstarch	9	68 ¹
Alcohol extract of ground wheat	..	See text

¹ Including the wheat extract.

In a second series of experiments the same method of testing was applied to muscle and liver of rats which had been reared from the age of 28 days to that of 64 to 66 days on diet 555, while parallel animals for comparison were fed through this same age period with diet 429, the composition of which is also shown in table 1.

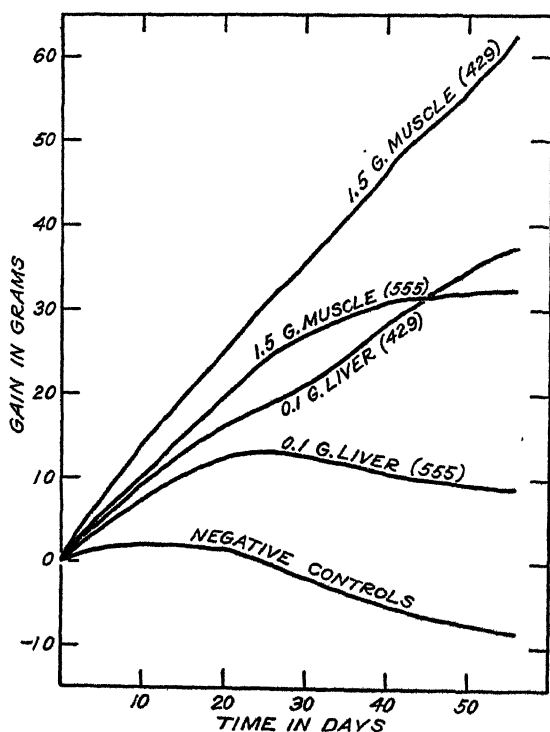


Fig. 2 Growth curves of experiments comparing the riboflavin contents of the tissues of animals from riboflavin rich diet 429 and riboflavin poor diet 555.

In the case of diet 555 the cornstarch carried an 80% alcohol extract of 60 gm. ground whole wheat to every 100 gm. of the air dry diet. This contains practically no riboflavin, and provides enough of all other water soluble vitamins needed by the rat for the amount of growth involved in our ordinary measurements of riboflavin values.

From figure 2 it will be seen that the tissues of rats reared on diet 429 (excellently adapted to all the nutritive requirements of rats) supported good growth consistently throughout the 8-week test period; while the results of feeding equal amounts of the corresponding tissues of rats reared on diet 555 (deficient in riboflavin) showed that the tissues of these latter rats contained distinctly less riboflavin than those of the rats whose food had furnished more of this substance.

DISCUSSION

The data just mentioned show also that the tissues of the animals from riboflavin deficient diet resulted in less consistently sustained growth. This latter finding may be explained in either of two ways. 1) As all of the tissues fed were highly relished by the test animals it may be that the feeding of even the riboflavin poor tissues stimulated a temporary increase in weight of the test animals somewhat greater than that properly attributable to their riboflavin intake alone. 2) It is equally conceivable that the early part of the weight curve is the valid measure of the riboflavin content of the tissues fed, and that the later flattening of the curve is due to the developing shortage of a second growth limiting factor of which the tissues of the diet 429 rats furnished more than did those of the rats from diet 555, while the tissues of the test animals contained enough for the support of their growth for a short time only, after which the curve flattens because of shortage of this further factor. This would be in line with the experience of Coward, Key and Morgan ('29) and of Chick and Copping ('30); and so our 'further factor' may be identical with the Coward factor, or Chick and Copping's 'Y factor' or both. It may also be identical with vitamin B₆. Of this, Copping ('36) finds only the lesser part extracted by 80% alcohol from ground cereals, so that in our diet 555 there may have been sufficient vitamin B₆ for only limited growth.

As present knowledge does not permit a confident choice between the foregoing alternatives, we have not a precise

quantitative measure of the riboflavin content in the tissues of the diet 555 rats; but it is clearly established that the level of riboflavin intake does exert a considerable influence upon the riboflavin concentration in the muscles as well as in the liver.

While riboflavin, like vitamins A, B₁ and B₆, occurs in greater concentration in the liver than in the muscles, the quantitative relationship varies widely. Sherman and Boynton ('25) found 200 to 400 times as much vitamin A per gram of liver as of muscle, while for the same species on the same diet Brodie and MacLeod ('35) found only a tenfold higher concentration of vitamin B₁, and we here find only from ten to twenty times as high a concentration of riboflavin in the liver as in the muscles. Working with a different species (beef) György ('35) likewise found for riboflavin a concentration in liver about fifteenfold higher than in muscle, while the concentration of vitamin B₆ was about one-third as high in muscle as in liver. The very low vitamin A content of muscle has not been found to be measurably influenced by the level of intake of this vitamin in the food, while this does have a very great influence upon the amount found in the liver, apparently because any surplus absorbed from the food is chiefly laid by in the liver, presumably in relatively passive storage. In the present experiments the level of intake of riboflavin has influenced the concentrations in the liver less and in the muscles more than was the case with vitamin A. This is consistent with the view that relatively less of riboflavin than of vitamin A is laid away in passive storage in the liver, and that relatively more of whatever surplus the food furnishes is distributed to the tissues generally, and raises their riboflavin content. In the experiments of Brodie and MacLeod ('35) increased intake of vitamin B₁ seemed to increase the concentration of this vitamin in muscle, liver, kidney and brain in about the same relative proportion. Thus there is some measure of similarity in quantitative distribution between the two water soluble vitamins, B₁ and riboflavin, as contrasted with the fat

soluble vitamin A. It also appears that the tissues tend, under stress of shortage to conserve their riboflavin more retentively than their vitamin B₁.

The findings for riboflavin as compared both with those for vitamin A and for vitamin B₁, tend therefore to confirm the general concept of riboflavin as an imperatively essential constituent of the tissues generally; and which when liberally supplied by the food reaches distinctly higher concentrations in the tissues than when the intake is of only minimal adequacy.

The present experiments might also be interpreted to support the view that muscle, liver, and milk (and Osborne and Mendel's 'natural protein-free milk') have special growth promoting value either because of factors not yet fully known or because of supplementary relations which exist among known factors but have not yet been fully explored. This is consistent with the differences between the growth curves shown in figure 2 as supported by feedings of corresponding tissues from rats differently fed, whichever of the alternative interpretations above suggested is considered the more probable.

We have no desire to increase the number of postulated nutritional essentials in the vitamin B complex. Rather it is to be hoped that these observations may ultimately be so integrated with those made in connection with the Coward factor (Coward, Key and Morgan, '29), factor Y (Chick and Copping, '30), vitamin B₆ (György, '35; Halliday and Evans, '37), vitamin H (Booher, '37), and the more detailed current postulates of the California, the Missouri, and the Wisconsin investigators, as to aid in the formulation of a clearer and more unified hypothesis as to the number and properties of the relatively heat stable, water soluble vitamins which function in mammalian nutrition.

SUMMARY

The primary purpose of this work was to determine the relative concentration, in the chief tissues of a presumably representative omnivorous mammal, of the nutritional factor which has been successively known as vitamin G, lactoflavin and riboflavin.

The tissues fed in the main series of experiments were from rats which had been reared on the same diet as in the case of previous studies of distribution of vitamin A. With riboflavin as with vitamin A the concentration in the liver was higher than in the skeletal muscle, and the concentrations in heart and kidney were intermediate; but the distribution of riboflavin shows a much less pronounced quantitative difference than was found in the case of vitamin A; for of vitamin A the concentration was 200 to 400 times as high in the liver as in the muscle, while of riboflavin the concentration in the liver was only ten to twenty times as high as in the skeletal muscles of the same animals. So far as may be judged from smaller numbers of measurements, the concentration of riboflavin was about tenfold higher in kidney, fivefold higher in heart, two- to threefold higher in brain and in spleen, than in skeletal muscle of the same well-nourished animals.

The level of dietary intake of riboflavin is found to influence the concentration of riboflavin in the body tissues generally.

In neither type of experiment were the differences great enough to indicate any such large storage capacity for riboflavin as the body possesses for storing vitamin A in its liver.

The bearing of these findings (and of others reported in the text) upon current concepts regarding the numbers and functions of the nutritionally essential factors of the vitamin B complex are discussed.

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CALCIUM DEFICIENCY AND INTESTINAL STASIS ¹

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FOUR FIGURES

(Received for publication July 1, 1937)

When young or half-grown rats are fed a diet deficient in minerals, their lower intestines become dilated and overloaded with fecal material and the excretion of barium or carmine is abnormally prolonged (Robertson and Doyle, '35). The addition of calcium to the mineral deficient diet almost entirely prevents this stasis, and does so completely if potassium is added as well (Robertson, '37). In addition we found that if children were fed a diet low in calcium and potassium, 75% of them became definitely constipated. We were therefore interested to see if a diet deficient in calcium alone or with an associated deficiency of potassium would lead to intestinal stasis and dilatation in rats, and to decide this question the following experiments were carried out.

The diets used in these experiments were similar to those used formerly, but the adequate diet contained 3.5% of Hawk and Oser's ('31) salt mixture, instead of Osborne and Mendel's ('18) mixture, and also the 1% NaCl was omitted. To make a diet deficient only in calcium, the calcium salts were left out of the Hawk and Oser mixture. When 1.8% of this special mixture was put in the diet, the rats received as much of the other cations as the adequate controls, but the P in the diet was reduced from 0.33% to 0.25%. Similarly the potassium salts were omitted in making the diet deficient in potassium

¹ An excerpt from a thesis submitted in conformity with the requirements for the degree of doctor of philosophy in the University of Toronto.

and in this the P was reduced to 0.21%. In a similar way the potassium and calcium salts were omitted to give a diet deficient both in potassium and calcium. In this diet no P was present in the salt mixture. As there was some Ca, K and P in the rest of the diet, all of the diets were only partially deficient in these elements.

Shortly after weaning, litters of rats were divided into five groups and fed the four diets outlined above and also the mineral deficient diet which contained the same basic

TABLE 1

Excretion of barium by rats fed various diets. Each figure is the average of from twenty to thirty-four rats

DIET	BARIUM IN FECES (DAYS)	
	X-ray determination	Chemical determination
Adequate	4.1	13
Adequate without K	3.7	14
Adequate without Ca	9.0	20
Adequate without Ca and K	10.0	21
Mineral deficient	9.0	20

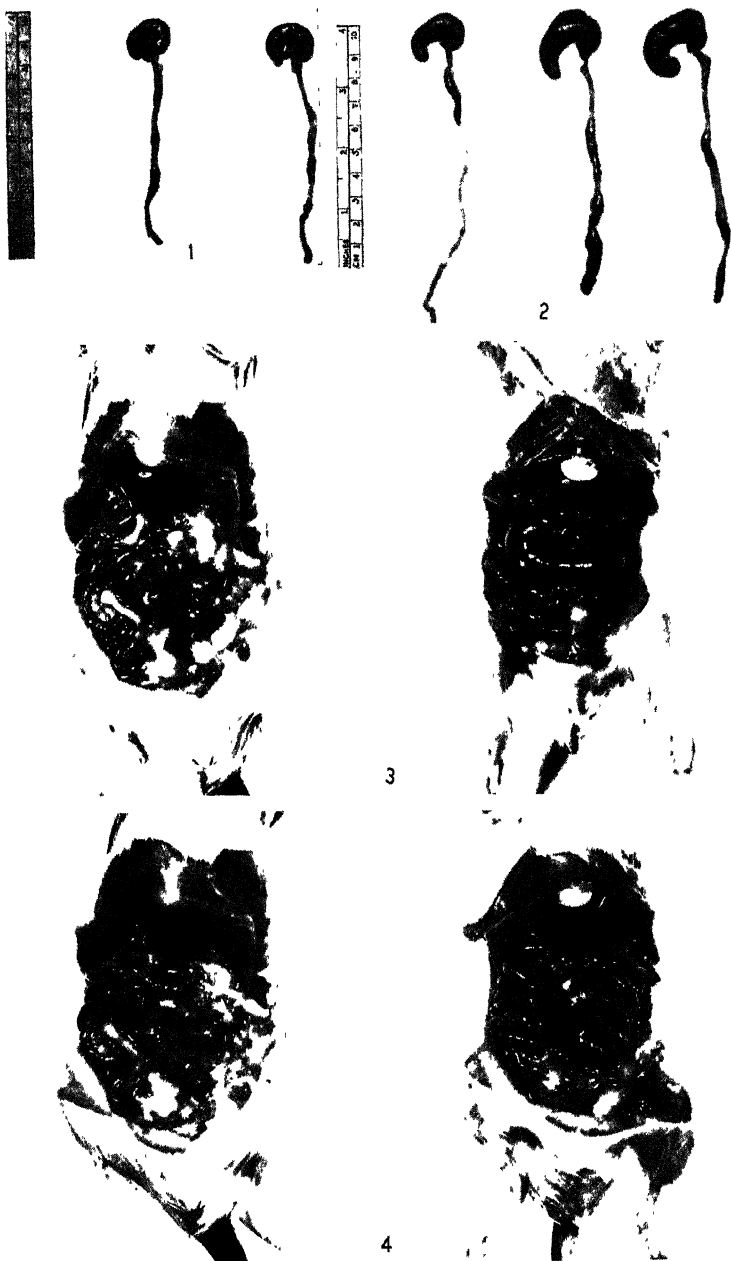
ingredients (cornstarch, casein, Crisco, dried brewer's yeast and cod liver oil) but no salt mixture. For brevity the diets were known as adequate, adequate without potassium, adequate without calcium, adequate without calcium and potassium, and mineral deficient.

Fig. 1 Cecums and colons of rats fed adequate diet (left side) and adequate diet deficient in potassium (right side). No difference in size can be seen. The rats were of the same age and weight.

Fig. 2 Cecums and colons of rats fed the adequate diet (left side), adequate diet deficient in Ca (center), and adequate diet deficient in Ca and K (right side). Note the larger cecums and colons in the rats deficient in Ca or Ca and K. The body weights of the rats were from 106 to 110 gm.

Fig. 3 Abdominal contents of rats of the same age fed adequate diet (left side), and adequate diet deficient in calcium and potassium (right side). The cecum, which is the stomach-shaped organ is much larger in the deficient animal and contains darker material. The adequately fed rat weighed 175 gm., the deficiently fed 113 gm.

Fig. 4 Abdominal contents of rats of the same age fed adequate diet (left side) and mineral deficient diet (right side). The cecum is much larger in the deficient animal, despite its much smaller body weight. The deficient rat weighed 125 gm., the adequately fed 175 gm.



After 5 to 7 weeks on the diets the rats were fed 1 dose of barium sulphate and the feces from each rat were then collected daily, and x-rayed and analyzed chemically to determine whether barium was present, using the methods described previously (Robertson, '37). As shown in table 1, the rats fed any of the diets deficient in calcium, no matter whether it was the mineral deficient, the adequate without calcium, or the adequate without calcium and potassium, all excreted the barium slowly. Possibly those fed the diet deficient in both calcium and potassium were a little slower than the others. Those fed the 'adequate without potassium' diet excreted the barium as rapidly as the adequately fed controls. In a personal communication, Prof. M. Bodansky recently informed the author that he also had observed intestinal stasis in rats fed diets low in calcium.

The rats were killed after 3 months on the diets. The intestinal tracts of the adequate controls and those fed the 'adequate without potassium' diet looked normal. The lower ileum contained only a moderate amount of material, the cecum was not enlarged and contained grayish feces and there was relatively little, gray, firm, fecal material in the colon. The cecums and colons of two typical rats are shown in figure 1.

On the contrary, in the rats fed the diets deficient in calcium there was much more material in the lower ileum and it was darker in color. The cecums were very large and contained blackish, four-smelling material and the colons were dilated and contained more dark, soft feces. These findings are shown in figures 2, 3 and 4.

In many of the rats fed the 'adequate without calcium' diet, the fur on the lower abdomen and back legs was thin and short, and many were also seen to have tetanic convulsions, especially if they were handled. About 10% of these rats died before the end of the experiment.

In further experiments the effects of these diets on the calcium, inorganic phosphorus and protein in the serum are being investigated.

CONCLUSIONS

If young rats are fed a diet low in calcium (about 0.08%) with or without an associated deficiency of potassium, they develop marked stasis and dilatation in the lower intestines, and they excrete barium sulphate very much more slowly than the adequately fed controls.

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BODY COMPOSITION AS A FACTOR GOVERNING THE BASAL HEAT PRODUCTION AND THE ENDOGENOUS NITROGEN EXCRETION

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ONE FIGURE

(Received for publication June 30, 1937)

The heat production of warm-blooded animals is generally believed to be proportional to approximately the two-thirds power of body weight. It is also commonly assumed that animals with an excess of adipose tissue have a lower basal metabolic rate (B.M.R.) per unit body weight than those containing less fatty tissue. Recent work would seem to indicate that the endogenous urinary nitrogen excretion is proportional to the basal metabolic rate. If such were the case then the same laws that govern the basal metabolic rate should likewise govern the endogenous nitrogen excretion.

Brody, Proctor and Ashworth ('34) have compiled a considerable amount of data from the literature showing the following relationships to hold for adult animals:

$$\begin{aligned}Q &= 70.5 M^{0.73} \\N &= 146. M^{0.73}\end{aligned}$$

where Q is basal metabolism in Calories per day, M is body weight in kilograms, and N is endogenous urinary nitrogen in milligrams per day. If we disregard the difference in exponents and divide the second equation by the first we find that 2.07 mg. endogenous nitrogen are excreted for every Calorie of basal heat produced. Smuts ('35) found the above

¹ Alexander Brown Coxe Fellow, 1935-1936.

constant to be 2.004 ± 0.015 . Using these values both the above papers propose to estimate the maintenance protein requirement of an animal from its basal heat production.

Whether such a relationship can be used for growing animals has been questioned by Ashworth ('35 a). Rats of weaning size were found to excrete less than 1 mg. endogenous urinary nitrogen per Calorie of basal heat produced while under the same conditions adult rats excreted 1.5 mg. nitrogen per Calorie. The object of the present investigation was to learn whether the body composition of the animal might affect independently the endogenous nitrogen excretion and the basal heat production, thus accounting for some of the variation which was found to exist in the ratio between them.

EXPERIMENTAL

Male albino rats ranging in body weight between 40 and 350 gm. were used. No attempt was made to produce animals with abnormal body compositions. We wished to determine only whether the normal variations of endogenous nitrogen excretion and basal metabolism could be accounted for by normal variations in the composition of the body. The animals used were given a diet capable of producing rapid growth (Smith and Smith, '34) from weaning until the time at which their endogenous nitrogen excretion was to be determined. They were then given a nitrogen-free diet composed of 58 parts dextrin, 10 sucrose, 4 Osborne and Mendel salts, 10 butterfat, 15 Crisco and 3 cod liver oil. The nitrogen-free diet was supplemented daily with 0.1 cc. of a tikitiki preparation containing 50 international units of vitamin B(B₁) per cubic centimeter.

After a preliminary period of 4 days on the nitrogen-free diet urine and feces were collected for 5 additional days in a collection chamber described elsewhere (Ashworth, '35 b). Following the last day of excreta collection the rats were fasted 17 to 20 hours and their basal metabolic rate measured in an open circuit Haldane apparatus. Two trains of absorption tubes were used so one set could be weighed while the

other was used to ventilate the animal chamber. CO₂ production was measured for six periods ranging in length from 30 to 70 minutes depending on the size of the animal. The mean of the three lowest values for CO₂ production together with the R.Q. over the entire period of 4 to 6 hours was used to calculate the basal heat production.

Immediately after completing the basal metabolic rate measurement the rats were killed, hashed and dried in a vacuum oven at 70° until there was no further loss of weight. The carcasses were then partially extracted with ether, ground in a mortar and passed through a 1 mm. sieve. Samples were weighed out for the determination of nitrogen (macro Kjeldahl, using the boric acid modification) and fat (Light, Smith, Smith and Anderson, '34).

DISCUSSION OF THE RESULTS

The original data are shown in table 1. It will be observed that there is considerable difference between the initial and final body weights of these animals. The initial body weights were recorded just before the animals were put on the nitrogen-free diet. While subsisting on such a diet prevention of body weight loss was not possible. This may be partially due to the fact that the rats had been accustomed to receiving a diet high in protein. Insufficient caloric intake is a criticism often made of similar results. We have kept food intake records to see whether the energy intake was large enough to prevent the loss of body protein for energy purposes.

It is difficult to estimate just what are the optimal caloric requirements of these animals. The small rat needs a proportionately larger excess above the basal for muscular activity than does the older, more sedate rat because, with an ad lib. intake of nitrogen-free food, the ratio of food Calories in excess of basal to basal Calories varied between 1.5 and 2.6 for rats weighing less than 100 gm., while it varied between 0.5 and 1.5 for larger rats. The minimal value of the above ratio indicates a caloric intake 50% greater than the basal metabolic rate requirement and would seem to be suffi-

cient for rats confined to small cages. No satisfactory demonstration could be made of a correlation between loss in body weight and the amount of nitrogen-free diet consumed although the results would indicate that the rats consuming the larger amounts of nitrogen-free diet, above a certain optimal level, lost weight more rapidly, a finding contrary to expectation.

TABLE 1
Original data

RAT NO.	BODY WEIGHT		AVERAGE FOOD INTAKE ¹	BASAL HEAT	NITROGEN		BODY COMPOSITION		
	Initial	Final			Urine	Feces	Dry weight	Total fat	Total N
	gm.	gm.	gm./day	cal./day	mg./day	mg./day	gm.	gm.	gm.
11	88	68	3.8	11.0	26.0	7.6	23.6	9.0	2.20
12	99	75	4.2	11.8	27.4	9.1	25.4	9.5	2.15
13	131	96	4.5	15.9	40.2	7.6	32.6	10.6	2.98
15	143	110	7.4	18.8	35.2	12.3	42.6	19.2	3.12
14	129	106	5.5	15.8	30.8	9.9	41.7	18.2	3.35
16	146	121	7.7	19.1	30.8	14.6	49.7	23.4	3.68
21	46	34	4.0	6.8	11.6	...	11.0	4.4	0.89
31	62	46	4.4	9.3	14.7	6.9	15.2	5.0	1.39
22	55	47	4.7	8.7	12.5	8.7	15.8	6.6	1.24
32	61	50	5.0	8.2	15.3	7.9	16.1	5.6	1.43
41	91	68	4.7	6.5	23.5	9.7	21.3	6.5	2.10
51	95	68	3.3	7.0	28.7	6.6	22.0	7.0	2.02
23	330	277	12.7	27.2	72.8	24.9	115.7	55.4	9.11
42	350	295	10.7	28.4	78.4	22.3	112.0	42.6	9.49
43	340	283	11.0	30.5	82.8	23.3	98.6	30.6	9.59
81	323	269	10.3	24.0	59.8	21.4	114.0	54.4	8.45
83	332	280	9.0	27.9	74.2	21.8	115.7	56.8	8.46
84	317	264	10.8	27.0	68.4	22.7	106.7	49.1	8.29
100	135	117	5.4	14.9	40.0	...	41.5	14.8	3.55
101	175	153	8.0	23.2	46.8	...	58.0	22.8	4.83

¹ Food intake during urine collection.

Since the rats were losing body weight at the rate of about 1% per day, it was considered advisable to determine the endogenous nitrogen excretion before the absolute minimal level was reached. There are many who believe that a preliminary period of 4 days on a nitrogen-free diet is all that is necessary to reduce the excretion of nitrogen to the minimum level in the rat. Ashworth and Brody ('33) have demonstrated that under the conditions here used rats do not reach their minimum level of nitrogen excretion in that length of

time. Smuts ('35) suggested that these rats may be excreting abnormally low amounts of nitrogen.

The probability that long periods of nitrogen-free feeding would produce changes in the body composition of the rat was considered likely. Such changes would be difficult to interpret when attempting to make any correlations with the minimal energy and nitrogen outputs of the animal. Therefore in this study the rats were kept on the nitrogen-free diet only 4 days preliminary to the collection of excreta.

TABLE 2

(1) $\log \text{Cal} = 0.648 \log \text{live wt.} - 0.126$ S.E. = 0.041	$r = 0.979$
(2) $\log \text{Ur N} = 0.853 \log \text{live wt.} - 0.208$ S.E. = 0.057	$r = 0.976$
(3) $\log \text{Cal} = 0.581 \log \text{dry body wt.} + 0.266$ S.E. = 0.042	$r = 0.978$
(4) $\log \text{Ur N} = 0.757 \log \text{dry body wt.} + 0.321$ S.E. = 0.072	$r = 0.962$
(5) $\log \text{Cal} = 0.622 \log \text{fat-free dry wt.} + 0.338$ S.E. = 0.039	$r = 0.981$
(6) $\log \text{Ur N} = 0.820 \log \text{fat-free dry wt.} + 0.400$ S.E. = 0.051	$r = 0.981$
(7) $\log \text{Cal} = 0.606 \log \text{body N} + 0.880$ S.E. = 0.041	$r = 0.979$
(8) $\log \text{Ur N} = 0.800 \log \text{body N} + 1.114$ S.E. = 0.054	$r = 0.979$
(9) $\log \text{Cal} = 0.734 \log \text{Ur N} + 0.073$ S.E. = 0.050	$r = 0.968$

Since surface area is commonly related to a fractional power of body weight and used as a unit of reference for the basal metabolic rate, power equations were used in the present investigation to correlate both basal metabolism and endogenous nitrogen with live body weight and with the various components of the body such as dry matter, fat-free dry weight and nitrogen content. Table 2 is a summary of these power equations in their logarithmic form together with their statistical constants. The equations were fitted by the method

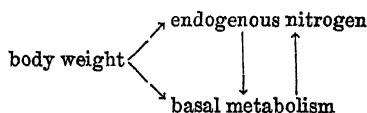
of least squares, the procedure being one of those outlined by Davenport and Ekas ('36). The wide range of body weight used probably accounts for the high correlation coefficients. The standard errors are, of course, in logarithm units.

One may readily observe that the components body nitrogen and fat-free dry weight offer the best standards of reference for both endogenous nitrogen and basal energy metabolisms.

No reason is apparent for the relatively low value for the correlation coefficient between endogenous nitrogen and the dry body weight of the animals (equation no. 4). When the body fat is removed there is a significant increase in the correlation coefficient as one may observe from equation no. 6.

If there were some fundamentally constant relationship between the endogenous urinary nitrogen excretion and the basal energy metabolism one would expect a higher correlation between them than between either of them and the various components of the body. Such is not the case. The correlation coefficient associated with equation no. 9 is, with the exception of that belonging with equation no. 4, the poorest of all. This fact would lead one to believe that endogenous nitrogen excretion and basal energy output are not proportional to each other.

In order to be entirely sure of this we have utilized the concept of partial correlation. Such a procedure is used when it is desired to eliminate some factor which may be affecting the correlation between the two factors under consideration. For example one may postulate that body weight is contributing to the correlation between endogenous nitrogen and basal metabolism as illustrated in the following diagram.



Partial correlation is then used to eliminate the effect of body weight. The formula used was as follows: (taken from Davenport and Ekas, '36; p. 109)

$$r_{12.3} = \frac{r_{12} - r_{13} \cdot r_{23}}{\sqrt{1 - r_{13}^2} \sqrt{1 - r_{23}^2}}$$

where the subscripts stand for the factors correlated. In the above example these are 1) endogenous nitrogen, 2) basal metabolism, 3) body weight.

The value of the partial correlation for the example given above, i.e., the correlation between endogenous nitrogen and basal metabolism holding the effect of the final live weight constant, was 0.27 ± 0.13 which is not significantly different from zero when its probable error is taken into consideration. Likewise when the effect of the various body components are eliminated by partial correlation the net correlation between endogenous nitrogen and basal metabolism remains without significance.

By supplementing the data of table 1 with those taken from a previously published paper (Ashworth, '35 a) the partial correlation of endogenous urinary nitrogen with basal metabolism holding live weight constant could be studied in more detail. Values for the original correlation coefficients were

$$\begin{aligned}\text{basal Calories: body weight} &= -0.946 \\ \text{endogenous nitrogen: body weight} &= -0.939 \\ \text{basal Calories: endogenous nitrogen} &= -0.871\end{aligned}$$

The correlation of heat production with endogenous nitrogen is significantly less than the correlation of either with body weight. When this latter correlation is corrected for the effect of body weight by partial correlation the resulting net value is -0.16 which shows a tendency toward an inverse relationship except that it is not large enough to be significant for the number of animals used (130 in this case).

Exponential equations for the first two of the above correlations were found to be:

$$\begin{aligned}\text{Calories} &= 0.540 \text{ body weight}^{0.720} \\ \text{endogenous nitrogen} &= 0.272 \text{ body weight}^{0.983}\end{aligned}$$

The large difference in exponents is quite significant in showing the absence of parallelism between the basal heat production and the endogenous nitrogen excretion of the growing rat. Similar differences are noted in table 2.

When the basal heat production of these animals is plotted directly against the endogenous urinary nitrogen excretion,

the curve instead of being linear in character is sigmoid. The shape of the curve is very similar to that of a typical body weight growth curve, or that of an autocatalytic reaction. Another way of presenting the same relationship is shown in figure 1. There the ratios of endogenous nitrogen excretion to basal heat production are plotted against the body weights of the animals. In order to make the trend more evident it has been smoothed out by the method of moving averages. The dotted line indicates how the trend should appear if 2 mg.

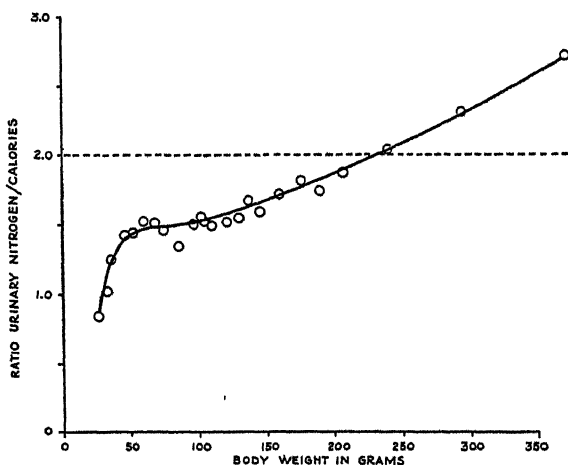


Figure 1

of endogenous nitrogen were excreted for every Calorie of basal heat produced, the thesis advanced by previous investigators. It is evident from figure 1 that there is no proportionality between the minimum requirement for protein and for energy in the growing rat.

In conclusion it appears then that the endogenous urinary nitrogen excretion and the basal energy metabolism of growing rats are not proportional to each other and that variations in body composition do not account for this lack of proportionality.

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THE ARREST OF NUTRITIONAL CATARACT BY THE USE OF RIBOFLAVIN ¹

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TWO FIGURES

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A recent report from this laboratory (Day, Darby and Langston, '37) described experiments which showed that the cataract which results from 'vitamin G' deficiency may be prevented by means of pure riboflavin,² either natural or synthetic. Since that report was written, rats have been kept for more than a year on a flavin-deficient diet, supplemented with 90 or 120 micrograms of riboflavin weekly, without showing cataract, keratitis or alopecia. Those animals have reached maturity, although growth proceeded at a somewhat subnormal rate on such levels of riboflavin feeding. Those experiments would seem to prove the identity of the rat cataract-preventive vitamin with flavin. The final test of this identity, however, would be experiments planned to show whether or not the development of cataract could be arrested by administration of pure riboflavin. This paper reports such experiments.

¹ Research paper no. 513, journal series, University of Arkansas. Read before the Division of Biological Chemistry of the American Chemical Society in Chapel Hill, April 13, 1937.

² The term riboflavin is used in this paper to mean the substance described as 6, 7-dimethyl-9-(*d*-1'-ribityl)-isoalloxazin (lactoflavin), in conformity with the terminology accepted by the Council on Pharmacy and Chemistry of the American Medical Association ('37).

In order to prevent confusion, it should be stated here that cataract in rats resulting from flavin deficiency is apparently quite different in etiology from the cataract which results from lactose or galactose feeding (Mitchell, Merriam and Cook, '37). The two types of cataract may be distinguished with the ophthalmoscope in the hands of a skilled observer. One type must be regarded as due to the deficiency of an essential substance; the other apparently results from the presence of a substance which, in high concentrations in the rat body, is harmful to lens tissue.

EXPERIMENTAL PROCEDURE

Our flavin-deficient diet, method of caging and care of animals, stock diet, and experimental procedure in flavin experiments with rats have been repeatedly described (Day, Darby and Langston ('37) and references cited therein). Two features of our technic might be pointed out as probably being especially important, however. The casein and other constituents of our experimental diet are flavin-free according to the most rigid tests available at the present time. The young rats are started on experiment when they are 21 days of age, at which time they weigh between 30 and 45 gm. each.

In the series of experiments to be reported in this paper, forty-three rats from six litters were given the flavin-deficient diet (no. 625). Weekly weight and food consumption records were kept, and the eyes were examined with an ophthalmoscope at weekly intervals or oftener. When early cataractous changes appeared in the eyes of the rats, certain of the animals from each litter were given riboflavin, while others were retained as controls.

The synthetic riboflavin used in this experiment³ was supplied to us in sterile ampules, each containing 1 mg. of riboflavin in 2 cc. of solution. Preliminary experiments indicated that it was sometimes difficult to get the deficient rats to consume the solution quantitatively. Consequently, in the experiments here reported, the flavin solution was administered by injection into the leg muscles. Each animal received

³ Supplied by the Winthrop Chemical Company.

0.24 cc. of the solution, equivalent to 120 micrograms of riboflavin, twice weekly. Twenty-five of the forty-three rats were given injections of riboflavin.

Although attention has previously been called to this fact, it is important to point out here that the cataractous changes in the lens of the eye in flavin deficiency are usually accompanied by, and sometimes preceded by, a generalized ophthalmia and dense opacities in the cornea (keratitis). This ophthalmia and keratitis make it sometimes difficult to see opacities in the lens (cataract) in their early stages. It is obvious that if administration of flavin were deferred until unmistakable evidence of cataract appeared—that is, until mature cataract developed—it would be futile to attempt to arrest the progress of the lens changes. Consequently, in our attempt to start the administration of flavin while cataract development was in its incipient stages, some errors of judgement were made. In some cases the flavin injections were started so late that the cataract went on to maturity. In other cases it was found that, after the animal had resumed growth and the keratitis cleared, no lens opacities were present. This was conceivably due to a 'cure' of cataract—a regression of the lens opacities. It is more probable, however, that no cataract had ever been present.

RESULTS AND DISCUSSION

Eighteen control animals received no supplement of riboflavin. Two of these animals died during the first few weeks of experiment (twenty-ninth and thirty-second days) without exhibiting cataract. Of the remaining sixteen control animals, thirteen (81%) exhibited cataract, and the average time of appearance was 52 days. The cataract proceeded to maturity in twelve rats (75%), at an average time of 67 days. It is therefore apparent that in this series of rats, as in similar groups previously reported from this laboratory, cataract appeared in a high percentage of those animals receiving an unsupplemented flavin-deficient diet, and the cataract proceeded to maturity in almost all such animals. The average survival of the sixteen control rats was 74 days.

Table 1 gives a summary of the data concerning the animals given riboflavin. Twenty-five rats were in this group, and for the reasons explained above, it was found as the experiment progressed that six of the rats did not have cataract. Of the nineteen rats showing lens changes, cataract proceeded to maturity in both eyes in two rats. By 'maturity' we mean opacities so marked that the eye appeared white by the most casual observation, and without the use of an ophthalmoscope or other instrument. In each of six other rats the cataract proceeded to maturity in one eye, but its progress was definitely arrested in the other eye. Cataract was arrested in

TABLE 1

Data on the arrest of cataract in rats given intramuscular injections of 120 micrograms of riboflavin twice weekly after early cataractous changes had appeared

	NUMBER	PER CENT OF TOTAL	PER CENT OF THOSE HAVING CATARACT
Arrested, both eyes	11	44	57.9
Arrested, one eye	6	24	31.6
Total arrested	(17)	(68)	(89.5)
Not arrested	2	8	10.5
Both eyes clear	6	24	
Total	25	100	100.

both eyes of the remaining eleven rats. Cataract was therefore definitely arrested in one or both eyes of seventeen out of nineteen rats with cataract (89.5%), and proceeded to maturity in both eyes of two of the nineteen rats (10.5%). In the rats with arrested cataract, no change in the condition of the lens can be seen now after 4 months.

Upon the administration of flavin there was an immediate and pronounced growth response (fig. 1). Where alopecia had been evident, new hair appeared. Ophthalmia cleared up rapidly, and keratitis eventually disappeared. During the period of rapid growth scabby ulcers appeared upon the head and body of many of the rats. The exact significance of this is obscure, but we have observed the same phenomenon whenever a source of flavin (as yeast) has been given rats which had

been deprived of the vitamin for an extended period. It appears to be a response to the feeding of flavin, and not an evidence that a deficiency of another substance has been superimposed.

The condition in the lenses with arrested cataract took several forms. In a number of eyes the entire lens was found to be clear except for a patch of opacity near the periphery

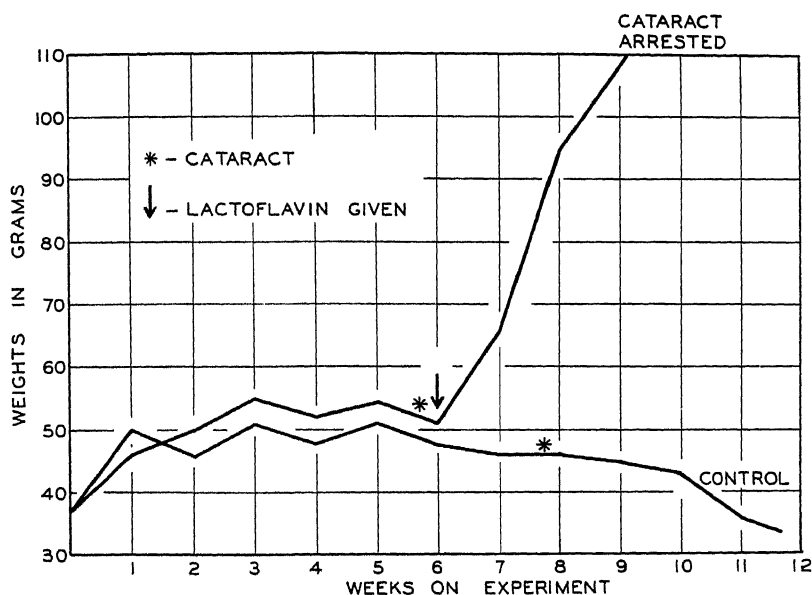


Fig.1 Weight curves of typical experimental animals. After early cataractous changes were seen in the eyes, 120 micrograms of riboflavin were given twice weekly to certain animals. Control animals were kept on the flavin-deficient diet until death.

of the lens, frequently in the side toward the ear. In other eyes it took the form of diffuse opacities throughout the lens. As seen through the ophthalmoscope these would frequently look like shattered 'shatterproof' glass. Such eyes would appear quite normal to the unaided eye in normal illumination. However, if carefully observed in a darkened room by the light of a small pocket flashlight, a snowy mass could be seen in the eye.

In one or both eyes of certain animals the arrested cataract took a third form. The lens opacities proceeded to maturity in the center of the lens, with a zone of less dense opacities surrounding this nuclear cataract, and a zone of clear lens tissue at the periphery of the lens. Figure 2 shows an enlarged photograph of a normal eye, and of an eye exhibiting arrested cataract of this type. Although it is only conjecture, there appears to be a very simple and reasonable explanation for this. In these cases of nuclear cataract with clear surrounding lens tissue, the central cataractous portion is about



Fig. 2 Enlarged photographs of eyes of two rats. Left, normal eye. Right, cataract arrested with riboflavin; in the lower center of the lens mature cataract (white) is evident. Surrounding this mature cataract is a zone of less dense opacities, and outside of this zone clear lens tissue is seen. The highlights on the eyeball are reflections from the flash-lamp and should be ignored.

the size that the entire lens was at the time that flavin administration was started. Since there is no mechanism for the repair or replacement of badly damaged lens fibers, cataract existing at the time of flavin administration would persist as a mass of opacities in the center of the lens. If we postulate that in these eyes the lens epithelium had not been destroyed, it would be possible, upon growth of the lens induced by flavin administration, for the lens epithelium to lay down normal lens fibers around this cataractous tissue. Such is apparently what happened in a number of cases. The cases of arrested cataract with diffuse opacities throughout the lens are less easily explained.

In 1934 we reported that nutritional cataract had been arrested by the feeding of milk powder to rats with early cataractous changes (Langston and Day, '34), and the action of the milk powder was ascribed to its 'vitamin G' content. It is now possible to reinterpret those data in terms of the riboflavin contained in the milk powder.

From the experiments reported in this and foregoing papers from this laboratory (Day, Darby and Langston, '37), it is now possible to conclude quite definitely that, with our diet and selection and care of experimental animals (rats), flavin deficiency results in cataract in a large percentage of cases. Furthermore, such cataract can be prevented by the feeding of pure riboflavin, and the progress of cataractous changes can be arrested by the timely administration of riboflavin to rats with incipient cataract. These experiments furnish clear evidence that riboflavin is a specific cataract-preventive substance for the rat.

SUMMARY

Young albino rats were given a diet deficient in flavin and their eyes were examined at frequent intervals with the ophthalmoscope. Of sixteen control animals receiving the deficient diet without supplement, thirteen (81%) developed cataract at an average time of 52 days. The cataract proceeded to maturity in twelve (75%) of these rats, at an average time of 67 days. The average survival was 74 days.

When early cataractous changes were evident, twenty-five rats were given intramuscular injections of riboflavin in doses of 120 micrograms twice weekly. The animals rapidly increased in weight, new hair appeared on those rats with alopecia, and keratitis slowly cleared up. In eleven of these rats cataract was arrested in both eyes. In each of six other rats the cataract proceeded to maturity in one eye, but its progress was definitely arrested in the other eye. The cataract proceeded to maturity in both eyes of two rats. Six rats were found to have clear lenses in both eyes after the keratitis cleared up. It is thus apparent that the progress of cataract

development was arrested by riboflavin administration in seventeen out of nineteen animals exhibiting cataract (89.5%). These data furnish additional evidence that flavin is the cataract-preventive vitamin.

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THE INFLUENCE OF SEX ON IRON UTILIZATION IN RATS ¹

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ONE FIGURE

(Received for publication July 6, 1937)

The possibility of a sex difference in utilization of iron was suggested first by Mitchell ('32) and very soon afterward by Rose and Kung ('32). Mitchell observed that at weaning time there was a slightly higher hemoglobin concentration in the blood of male than of female rats. She had records of 283 males having an average hemoglobin of 8.2 gm. per 100 cc. of blood and of 291 females averaging 9.2 gm. She found also that the average length of time required to induce severe anemia was 1.1 weeks longer for a group of 133 females than for the corresponding group of 129 male litter mates. Rose and Kung ('32) found that to induce anemia by removal of blood was more difficult in case of females than of males. In a group of twenty-three females and fifty-five males, there were twenty-two animals from which more than the average amount of iron had to be removed by bleeding to accomplish their purpose and of these eighteen were females. These studies raised the question as to whether there was some characteristic of the female which caused her to utilize iron more economically than the male. Mitchell suggested that a better prenatal store of iron might explain the tendency of females to become anemic more slowly than the males on

¹Based on a thesis presented by Helen Jackson Hubbell in partial fulfillment of the requirements for the degree of doctor of philosophy.

a diet poor in iron. However, Rose and Kung, having carried their animals to a normal hemoglobin level and then depleted them again, thought the observed differences might be accounted for by the greater rate of growth of the male and the larger food consumption in consequence. Smith and Otis ('37) have, since this work was completed, published a study of equal numbers of male and female rats fed the same amount of iron at the end of a depletion period and followed for 6 weeks thereafter to observe any differences in their gains in hemoglobin. On various levels of iron intake up to 0.2 mg. daily, the females produced slightly more hemoglobin than the males. In their study, however, the iron intake of the females was not kept the same per gram of rat as that of the males.

There are but few analyses of the iron content of the bodies of normal rats reported in the literature. Smythe and Miller ('29) give the most complete data for different age groups but the number of females used is too small to determine averages for the two sexes separately.

The aim of the present investigation has been to study the utilization of iron after ruling out the influence of the inherent sex difference in growth by feeding iron and copper in dosages carefully adjusted to changing body weights throughout the whole period of investigation. The general plan was to reduce young male and female rats to a uniform state of anemia by milk feeding, then to feed the depleted animals iron and copper supplements, the dosage of iron for each rat being calculated for each week on the basis of its body weight, and that of copper being sufficient to insure full utilization of the iron for hemoglobin formation. When the hemoglobin was practically uniform at 14 gm. per 100 cc. of blood the bodies were analyzed for iron and sexes compared as to the percentage of iron found.

METHODS

Well-matched male and female rats 21 to 27 days old, weaned from mothers fed Sherman's diet B, consisting of one-third whole milk powder and two-thirds whole wheat plus sodium

chloride and supplemented by lean beef, were placed in separate galvanized iron wire cages freshly treated with aluminum paint, having raised bottoms of $\frac{1}{2}$ inch mesh to prevent consumption of feces. All animals were weighed weekly and their consumption of milk was recorded. Quantitative hemoglobin determinations were made by the colorimetric method described by Exton and Rose ('33) using the Exton photoelectric scopometer ('32). Duplicate samples of 0.025 cc. of blood drawn from snipped tails were placed in 5 cc. of tenth normal hydrochloric acid and after standing 20 to 45 minutes were read in the scopometer.

When the animals had been depleted to between 4 and 5 gm. of hemoglobin per 100 cc. of blood, some were killed and reserved for iron analyses and the rest were fed dried whole milk supplemented by iron and copper, the amount of iron being adjusted weekly according to the body weight of each animal. During this period, hemoglobin determinations were made from the fourth week of iron and copper feeding until the hemoglobin reached approximately 14 gm. per 100 cc. of blood when they were killed and their bodies analyzed for iron. Most of the animals reached this level within 6 weeks. A few which did not were kept on the iron feeding through the seventh week. All of them reached a level of more than 13 gm. per 100 cc. of blood.

The bodies, carefully ashed as described by Rose and Kung ('32) were analyzed by the method of Rose, McCarthy, Blacker, Schattner and Exton ('36). The ash of each rat body was dissolved in hydrochloric acid and made up to 100 cc. so that the acidity of the final solution would be about 10%. For each determination 0.5 or 1 cc. of rat solution was placed in a small test tube and 3 cc. of water and 0.5 cc. of a normal solution of potassium thiocyanate were added. Three successive extractions of the resulting ferric thiocyanate were made with iron-free amyl acetate in portions of 4 cc., 3 cc. and 3 cc., gently tipping the test tube back and forth fifty times after each addition and then pouring the contents into a 30 cc. pear-shaped separatory funnel by means of which the aqueous layer

was withdrawn for the next extraction. The amyl acetate solution, collected in a 15 cc. centrifuge tube, was centrifuged for 1½ minutes to remove the last trace of turbidity before readings were made on the scopometer. Determinations were always made at least in quadruplicate and independent analyses were made by each of us. The delicacy of the method is indicated by some typical figures shown in table 1.

Since in some methods the reactivity of the iron is interfered with by the formation of pyrophosphates, a careful study

TABLE 1
Some iron analyses of rat bodies made at different times and in part by two analysts

ANALYST	RAT NO.	DATE	SCOPOMETER READING	MILLIGRAMS Fe PER RAT
H	XI M ₂	10/5	408	6.66
			410	6.75
		10/15	407	6.63
			408	6.66
H	XI F ₁	10/15	273	0.77
			273	0.77
		10/16	274	0.78
			275	0.80
H	II M ₂	3/22	343	4.73
			345	4.76
		10/12	345	4.76
			343	4.73
H	VII F ₁	9/28	395	6.26
			393	6.20
R	VII F ₁	10/24	395	6.26
			395	6.26
H	IV M ₃	9/18	399	6.36
			399	6.36
R	IV M ₃	10/23	400	6.40
			398	6.33
H	III F ₁	10/8	291	1.76
			290	1.74
R	III F ₁	10/9	290	1.74
			291	1.76

was made to be sure that there was no such interference in applying this method to rat bodies. A number of samples of rat ash solutions tested before and after boiling with hydrochloric acid for 1 hour with a reflux condenser gave practically identical results. As a further test pyrophosphate was added to one of duplicate samples of known iron content and both were then subjected to the same ashing process as the rat bodies and analyzed for iron. The results were in as close agreement as the quadruplicate analyses on either sample thus showing conclusively that under the conditions described there is no interference of pyrophosphates.

Some checks of the scopometer method were made by the Zimmermann-Reinhardt method, which has been used with success in this laboratory but which was not used in this study because it requires too large samples to permit more than one or two tests on the same rat body, whereas with the scopometer a dozen were sometimes made and fifty or more would have been possible. Analyses of four rat bodies agreed within 2 to 4% which is within the experimental error of the Zimmermann-Reinhardt method. In four other comparisons, the differences were slightly higher, but the average of the eight samples was a difference of 4.3%, and it must be borne in mind that only one sample was available for each Zimmermann-Reinhardt determination.

In case of milk, it was necessary to modify the method to avoid the interference resulting from the high concentration of salts, especially phosphates, in the solution obtained by dissolving the ash of 40 gm. of powdered milk or 300 gm. of fresh milk in hydrochloric acid and making it up to 50 cc. It was necessary to take 5 cc. of this solution for a determination. The acidity (already 12 to 15%) was increased by adding 2 cc. of concentrated hydrochloric acid, and the ferric chloride in solution was extracted three times with a mixture consisting each time of 5 cc. of ether and 1 cc. of acetone. A fourth extraction, tested with potassium thiocyanate, was always found to be iron-free and was discarded. These extracts were combined in a 25 cc. beaker and the ether and

acetone evaporated over warm water in a dust-free alberene hood in a protected inner room, under a tent of filter paper. Then 2 cc. of water was added to the residue, the solution was made slightly alkaline with a few drops of twice normal sodium hydroxide, neutralized with hydrochloric acid, using phenolphthalein as indicator, and again acidified with 2 drops of concentrated hydrochloric acid. Thereupon 0.5 cc. of normal potassium thiocyanate was added and extraction made with amyl acetate as already described.

The iron and copper were fed to the rats as ferric chloride and copper sulphate, respectively, the solutions being so adjusted that 1 cc. of ferric chloride solution contained 0.4 mg. of iron, and 1 cc. of the copper sulphate solution contained 0.1 mg. of copper. On weighing days the weekly allowance was calculated for each animal and from this total was subtracted the amount of iron and estimated copper consumed in the milk during the previous week. The supplements were fed three times a week. Each dosage was given in 5 cc. of reconstituted powdered milk.

THE DEPLETION PERIOD

Since the depletion period on powdered milk was found to average 32 days, an attempt was made to hasten matters by using fluid Guernsey milk obtained by direct milking into specially cleansed glass bottles. It was thought that the high fat content (5%) of the Guernsey milk might result in a higher calorie intake and hence more rapid gain in body weight. The fluid milk had no effect on the males and caused only a slightly more rapid decrease in the percentage of hemoglobin in the blood of the females. For twenty-two males, eleven in each group, the average length of the depletion period was the same, 28 days. The average time for twelve females on powdered milk was 36 days and for fifteen females on fluid milk, 31 days. The average daily food intake in dry weight per gram of rat was 0.065 gm. for the males on powdered milk and 0.046 gm. for the males on fluid milk. For the females it was 0.070 gm. per gram of rat on powdered milk

and 0.052 gm. on fluid milk. The average hemoglobin for the eleven males in the powdered milk group was 4.3 gm. per 100 cc. of blood and in the fluid milk group, 4.0 gm. In the case of the females, the group on powdered milk averaged 4.6 gm. and the group on fluid milk 4.3 gm.

Determination of the iron content of the bodies of seven males and ten females depleted by milk feeding to from 3.4 to 4.9 gm. of hemoglobin per 100 cc. of blood gave the same results for both sexes, 0.013 ± 0.0004 mg. per gram of body weight. Since the animals which were fed iron, were depleted to the same level, namely under the same conditions, it seems safe to conclude that they, too, had a like iron content at the end of the depletion period. This is in harmony with the findings of Rose and Kung ('32) who found that animals depleted to between 5 and 6 gm. of hemoglobin per 100 cc. of blood had an average iron content of $0.0021 \pm 0.00003\%$.

Schultze and Elvehjem ('33) have called attention to the fact that if copper be given to anemic rats it will tend to stimulate the utilization of any reserve iron available for hemoglobin regeneration and so increase the hemoglobin of the blood. To make certain that under the conditions of this investigation all reserve iron was exhausted when the iron-feeding period began, 0.1 mg. of copper was fed daily to two litters of depleted animals. Of sixteen animals so tested, there were five males and two females that showed practically no change in hemoglobin after 1 week; two males and three females showed an increase of from 0.4 to 1.4 gm. per 100 cc. of blood during the first week but returned to the former level the next week; one male and three females showed increases in hemoglobin from 0.8 to 1.3 gm. per 100 cc. of blood during the first week, and remained at that level until chloroformed 3 weeks later. These very slight increases, being within normal variability, did not indicate any necessity for administration of copper during the depletion period to insure full exhaustion of iron reserves.

THE IRON-FEEDING PERIOD

There are no data in the literature regarding the iron requirement of rats on the basis of body weight, and therefore, it seemed advisable to make this study on three levels of iron intake, e.g., 0.0019, 0.0038 and 0.0057 mg. per gram of body weight per day. The copper intake was uniform for the three series, 0.00038 mg. per gram of rat per day.

In series A, the group on the lowest iron allowance, the total daily intake of iron per rat amounted to 0.14 to 0.28 mg. in the first week and to 0.26 mg. to 0.48 mg. in the last week. The copper ranged from 0.027 mg. to 0.056 mg. per rat per day for the first week. By the sixth week no animal was getting less than 0.045 mg. per day which more than covered the requirement as indicated by studies of Myers and Beard ('31) and of Hart, Steenbock, Waddell and Elvehjem ('28). In series B the minimum iron intake per rat per day ranged from 0.27 to 0.41 mg. the first week, and from 0.54 to 1.00 mg. the last week. The copper dosage was the same as for series A. In series C, the minimum iron intake per rat per day ranged from 0.41 mg. to 0.57 mg. in the first week and from 0.85 mg. to 1.64 mg. in the last week.

Analysis of the bodies when the animals had reached a hemoglobin value of approximately 14 gm. per 100 cc. of blood showed that in each series the amount of iron per gram of body weight was higher for the females than for the males. Of those in series A receiving 0.0019 mg. of iron per gram of rat per day, the seven males averaged 0.030 ± 0.0003 mg. per gram of rat and the eight females 0.034 ± 0.0005 mg.; of those in series B receiving 0.0038 mg. of iron, the eight males averaged 0.034 ± 0.0006 mg. and the nine females 0.038 ± 0.0007 mg.; of those in series C receiving 0.0057 mg. of iron, the four males averaged 0.033 mg. and the four females 0.037 mg. The differences are shown in figure 1 and the full data are given in table 2. In series A the difference between the means of the iron content of the males and females was 6.7 times the probable error of the difference; and in series B it was 4.4 times the probable error of the difference. The third series was not continued beyond the

study of eight animals because the differences between the sexes were in close agreement with those on 0.0038 mg. of iron. These differences between the sexes, expressed in percentage were 13, 12 and 11%, respectively, for the three series.

Even in the depletion period there was evidence that the females had greater reserves of iron than the males. In a group of sixteen rats equally divided as to sexes, there were three females but only one male failing to deplete to a hemoglobin level below 5 gm. per 100 cc. of blood in 7 weeks. Also the depletion periods of the females in the first two series

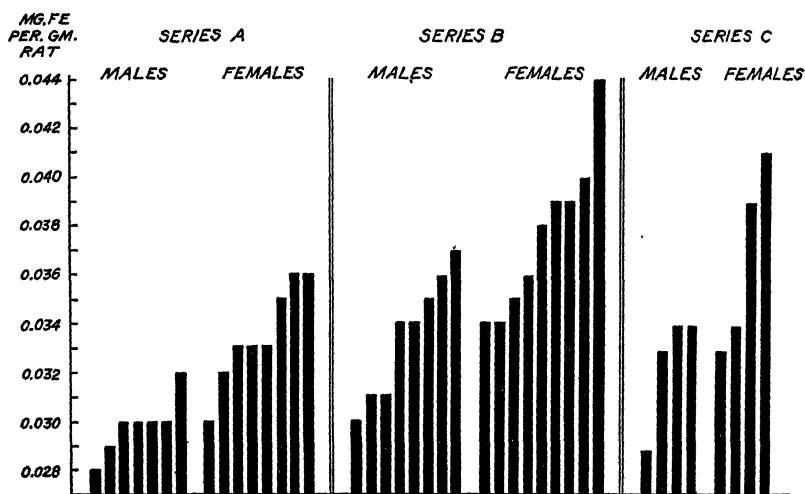


FIG. 1. IRON PER GRAM OF BODY WEIGHT OF INDIVIDUAL RATS

and in the group of anemic controls were, respectively, 24, 17 and 17% longer than those of the males.

Since there is, as shown by this study, a slight but consistent difference in the iron content of the bodies of male and female albino rats studied at different levels of intake, the question arises whether the female differs from the male in the method of utilizing the retained iron. Does the female store iron in the liver and other tissues to a greater extent than the male or does she produce more hemoglobin per gram of body weight? According to Elvehjem and others ('32), Josephs ('32), Cook and Spilles ('31) and Cunningham ('31) any absorbed iron,

MALES					FEMALES				
Iron-feeding period				Iron-feeding period				Iron content of rat	
Number of days	Weight at end	Hemoglobin at end	Gain in hemoglobin	Per gram rat	Number of days	Weight at end	Hemoglobin at end	Gain in hemoglobin	Per gram rat
Series A. Rats fed 0.0019 mg. Fe per gram rat per day									
49	245	14.8	10.2	7.41	49	183	13.7	8.8	0.036
42	265	14.9	9.9	7.61	49	165	14.0	9.5	0.033
40	233	13.5	9.6	6.26	49	191	13.8	8.5	0.035
49	221	14.6	10.8	6.35	49	154	13.4	9.1	0.033
35	187	14.3	10.0	5.34	49	154	14.2	9.3	0.033
42	223	14.2	9.7	6.29	48	178	13.2	8.7	0.032
48	241	13.7	9.8	6.95	48	182	13.7	9.2	0.030
					48	146	14.5	9.8	0.036
45	230	14.3	10.0	0.030 \pm 0.0003	49	169	13.8	9.1	0.034 \pm 0.0005
Series B. Rats fed 0.0038 mg. Fe per gram rat per day									
35	226	14.2	10.0	7.26	42	140	14.5	10.5	0.039
35	230	14.3	10.2	6.72	42	160	14.9	9.9	0.034
42	281	14.2	10.5	8.50	35	151	14.5	9.3	0.035
35	226	14.0	9.4	7.24	35	179	14.9	10.3	0.040
49	233	13.5	9.5	8.13	48	170	13.4	9.3	0.034
35	195	14.3	10.7	6.49	35	161	14.6	10.1	0.036
35	211	14.6	10.4	7.48	34	149	14.8	10.7	0.044
42	241	14.3	9.8	7.00	35	171	14.0	10.2	0.038
					35	152	14.9	10.1	0.039
39	230	14.1	10.0	0.034 \pm 0.0006	38	159	14.4	10.0	0.038 \pm 0.0007
Series C. Rats fed 0.0057 mg. Fe per gram rat per day									
42	272	15.0	11.8	8.56	42	160	14.5	10.4	0.034
42	222	12.5	8.5	6.03	41	175	13.3	9.0	0.033
40	217	14.1	10.1	6.97	42	149	13.5	9.9	0.041
42	200	14.7	10.6	6.69	41	173	13.9	9.0	0.039
41	228	14.1	10.3	0.033	42	164	13.8	9.6	0.037

whether obtained from the diet or stored in liver and spleen, is utilized for the production of hemoglobin when copper is fed.

The finding of Rose and Kung ('32), that more hemoglobin had to be withdrawn to make a female anemic than a male gave strong presumptive evidence of the hemoglobin formed, but the data of this study do not furnish a final answer to the question. An estimate of the total blood in the body of each rat was possible, using the formula of Chisholm revised by Hortar as cited by Donaldson ('24). Such an estimate showed that the females of each series had more hemoglobin per gram of rat than the males of their respective groups. This difference amounted to 11% for series A and 14% for series B, thus paralleling fairly closely the differences in iron content, 13 and 12%.

It is interesting to note in this connection that the influence of sex on the storage of body constituents other than iron has been reported in the literature for both calcium and vitamin A. Sherman and MacLeod ('25) reported that at all ages the percentage of calcium in females which had not reared young is higher than that in males of the same age receiving the same diet. Ender ('34), studying the vitamin A content of livers of bullocks and cows, concluded that there was four to five times as much of this vitamin in the liver of the female as in that of the male.

SUMMARY

The iron content of carefully matched young male and female rats depleted to approximately 4 gm. of hemoglobin per 100 cc. of blood had an iron content of 0.013 ± 0.0004 mg. of iron per gram of body weight.

The bodies of a group similarly depleted and then fed 0.0019 mg. of iron per gram of body weight until the hemoglobin reached 14 gm. per 100 cc. averaged for the males 0.030 ± 0.0003 mg. and for the females 0.034 ± 0.0005 mg. Another group fed 0.0038 mg. of iron per gram of body weight averaged for males 0.034 ± 0.0006 mg. and for females

0.038 ± 0.0007 mg. A third group fed 0.0057 mg. of iron per gram of body weight averaged 0.033 mg. for males and 0.037 mg. for females. The females stored on the average about 12% more iron than the males when the intake was the same per gram of body weight.

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A METHOD OF INCREASING PRECISION IN VITAMIN A ASSAY ¹

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The difficulties encountered in assaying foods and pharmaceutical materials for vitamin A are not adequately met, in the opinion of many workers, by the directions of the committee for revision of the U. S. Pharmacopoeia ('34). Richards ('35), for example, has presented a severe criticism of the plan of the test. In general practice too often, disconcerting variation has been the rule. The many rats failing to survive the long experimental period also has complicated results. While the recently recommended procedure of feeding carotene to one lot of an assay series has improved the reliability of the test, it involves the hazard of the unexplainable variations so often encountered in the response of the animals in the reference group itself.

Success in assay work has been attained in our laboratory only with the use of large experimental lots. With them, we have been able to obtain graded response at graded levels of feeding and to reproduce results at a specific dosage in consecutive years. Since 1933 we have been investigating the possibilities of increasing the accuracy of the assay by two methods: the shortening of the assay period, and the elimination of animals showing indications in the depletion period of erratic response to the test. The results have been fairly

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satisfactory. This report deals with the results of these experiments.

EXPERIMENTAL

During the fall, winter and spring months of 1932-1935, 557 standard test animals depleted of their bodily stores of vitamin A were fed quantitative graded supplements of carotene, canned tomatoes, or sweet potatoes, the level of dosage ranging from approximately 1.0 to 3.0 Sherman units daily. The carotene used was the provisional standard material first supplied by the League of Nations ('31).

The animals were of Wistar stock, strain A, inbred by brother and sister matings for sixty-five generations from representatives of the second litter. Average increments in weight at successive age intervals in these rats were the same from generation to generation and in composite samples of mixed generations from year to year (Timson, '32). Thus, a uniform average rate of growth appears to have been fixed by inbreeding.

Female rats only of our particular strain of animals are suitable for vitamin A assay work. Of the 557 females depleted of their bodily stores of vitamin A, 91% survived an experimental period of 8 weeks whereas only 58% of a group of 469 brother males lived throughout this period.

The test animals were taken from litters reduced to eight rats during the first week of life. When given the A-free ration, their mean weight was 43.5 gm., with standard deviation, 14.5 gm.

The constituents of the stock diet ² were kept as constant as possible in regard to vitamin A value from season to season. A supply of whole corn, sufficient to meet the needs of the

² The percentage composition of the basal stock diet was as follows: ground yellow corn, 64; linseed meal, 16; crude casein, 5; alfalfa, 2; NaCl, 0.5; CaCO₃, 0.5; yeast, 1.5; irradiated yeast, 0.5; wheat germ, 10. This diet was supplemented daily with liquefied Klim to which was added cod liver oil (1 teaspoon per quart) and trace minerals (2 cc. per quart of a solution containing KI, MgSO₄, K₂Al₂(SO₄)₃, and CuSO₄). In addition, the rats received 5 gm. of raw lean beef and 10 gm. of lettuce on alternate days.

laboratory for a 4-year period was purchased and stored, small quantities being ground as needed. Once every year, a supply of dried winter milk³ representing 1 day's run in the factory was purchased. Recorded data⁴ show that these procedures measurably reduced variability in the time required for exhaustion of bodily stores of vitamin A, especially from season to season.

The animals were housed individually in cages with raised wire bottoms. Care was exercised in maintaining the cleanliness of the cages, particularly of the water cups (Ewe, '32).

The basal ration contained the following ingredients: casein (vitamin A-free,⁵ 18%; hydrogenated lard,⁶ 22%; corn-starch, 56%; Osborne and Mendel salt mixture,⁷ 4%; 0.5 gm. of yeast,⁸ one-fifth of which was irradiated.⁹ The yeast was fed separately to each rat daily, thus insuring an adequate level of the B-complex in the diet after the rat voluntarily decreased its food intake.

Five-tenths gram of yeast per rat per day (approximately 8% of the diet) supplementing an otherwise adequate diet was shown to promote normal growth during the period of the experiment.¹⁰ The yeast contained no traces of vitamin A (Honeywell, Dutcher and Ely, '31), no lengthening of the depletion period being observed when the level of yeast was increased to 40%. The irradiation of part of the yeast furnished adequate vitamin D as shown by appropriate assays.¹¹ More uniform depletion records were obtained with

³ Klim, The Borden Co., New York.

⁴ Unpublished data, nutrition laboratory of the Foods and Nutrition Department, Iowa State College.

⁵ Crude casein, Wilkens-Anderson Company, Chicago, extracted four times (each extraction period, 1 hour long) with hot 95% alcohol, washed thoroughly between each extraction, and finally air-dried.

⁶ Clix, Cudahy Packing Co., Chicago.

⁷ J. Biol. Chem., vol. 32, p. 309, 1917.

⁸ Yeast Foam Tablet Powder, the Northwestern Yeast Co., Chicago.

⁹ Two hundred and fifty grams of yeast spread over an area measuring 12 × 16 inches was irradiated for $\frac{1}{2}$ hour, with frequent stirring, 18 inches from arc of a quartz mercury vapor lamp.

¹⁰ See footnote 4.

¹¹ We are indebted to Dr. B. H. Thomas of the animal chemistry nutrition subsection of the Iowa Agricultural Experiment Station, and to Dr. J. F. Edwards of the department of hygiene for these determinations.

the use of irradiated yeast than when D was supplied either by the irradiation of the animal or of the basal diet.¹²

Clix was used as the dietary fat because smaller increments of growth were observed in the depletion period with this fat in the ration than when Crisco was employed.¹² The use of fat in the diet prevented xerophthalmic symptoms due to irritation from food dust (Bauman and Steenbock, '34).

A normal rate of growth was induced by the addition of only 1.5 mg. of a standard cod liver oil to the A-deficient diet, evidence, we believe, that the feeding of the A-free ration produced an uncomplicated dietary deficiency inasmuch as the diet had already been proved adequate in vitamin D. None of the difficulties reported by Coward, Key and Morgan ('29) in a similar supplementation were experienced.

The rats were depleted of their bodily stores of vitamin A in 22.6 days (standard deviation, 10.0) which is approximately the time (23.6 days) that persistence of cornified cells in their vaginal smears was observed. Only the mere beginnings of xerophthalmia were present.

In order to meet Richards' criticism ('35), we gassed a group of animals that we considered adequately depleted and examined them for pathological lesions. A slight hyperemic condition of the intestinal capillaries and a trace of blood in the feces were the only abnormalities found. After 28 days, advanced xerophthalmia, chalky teeth, hemorrhage of intestinal capillaries and pus pockets in the ears and nose were noted in the animals examined.

Criteria for judging the exhaustion of vitamin A reserves were developed in this laboratory after 2 years of critical study because indices described in the literature (Sherman and Munsell, '25; Holmes, '32; U. S. Pharmacopoeia Committee of Revision, '34) were found somewhat too severe to apply in our particular experimental situation. These were: 1) maintenance of constant weight¹³ for a period of 5 days

¹² See footnote 4, page 105.

¹³ Constant weight is defined as that weight that does not alter more than ± 4 gm. from day to day. Such leeway in definition considers the effect of diurnal variation.

and incipient xerophthalmia;¹⁴ 2) maintenance of constant weight for 4 days and slightly advanced xerophthalmia;¹⁴ 3) severe xerophthalmia¹⁴ only; and 4) a drop in body weight of 4 or more grams sustained for 2 days. If any one condition prevailed, the animal was considered depleted. The interval from the time that the animal first began to show signs of vitamin exhaustion to the time when it was ready for supplementary feeding was called 'the critical period.' Accuracy of judgment was greatly improved when the practice of weighing and observing of the animals daily throughout the depletion period was initiated.

In the preparation of the test lots every effort was made to obtain representative random samples. At depletion, the individuals of a litter were distributed insofar as possible in the various lots representing an assay. A sufficient number of breeding animals was used to insure the placing of approximately fifteen animals in each lot within a period of 30 days. Complete records were kept in both the depletion and growth periods of the animals.

RESULTS ATTAINED WITH METHOD DESCRIBED

Since an earlier report from this laboratory (Irwin, Brandt and Nelson, '30), the most significant variations in routine procedure introduced were: change in indices for determination of depletion of bodily stores of vitamin A, offering of yeast separate from the basal ration, a tested dietary source of vitamin D, the use of females only in the test, and a standardization of the stock colony ration. The standard deviation from the mean gain (32 gm.) of a group of 349 animals fed sources of vitamin A for 8 weeks as reported by Irwin, Brandt and Nelson was 53 gm., in the 557 rats used in assay work in

¹⁴ A definite progression may be noted in the development of xerophthalmia (Steenbock and Wirick, '31). We recognize 3° of xerophthalmia in judging depletion, i.e., incipient xerophthalmia, characterized by a slight swelling of the eyelids or a small exudate in the corner of the eye; slightly advanced xerophthalmia in which an exudate extends halfway around a reddened eye; severe xerophthalmia in which the entire eye is surrounded by exudate and the lids are very swollen and inflamed.

the laboratory since then, the standard deviation was 22 gm. (mean gain, 35 gm.). Thus, the details of standardization described in the previous section were effective in improving the assay.

In table 1 is a summary of results from forty-nine tests involving 439 rats. The tests are grouped in ten lots according to the level and supplement fed. Analysis of variance of each lot (Fisher, '30) showed that the test differences were in no case significant. The ten lots, therefore, were each assumed to be homogeneous for gain. The 138 rats whose records are not included in table 1 fell into miscellaneous tests, many representing only one assay.

Coward, in 1932, found the standard deviations from the mean gains were fairly uniform over a large number of test lots and furthermore, that the standard deviation was independent of the mean increase in weight of the test group. We thereupon calculated the standard deviations of the gains made by the ten lots of rats during the 8-week test period. Although these groups had been given different levels of vitamin A, considerable similarity in the results was observed. All but one of the ten calculations fell within the range, 17 to 20 gm. (table 5). Thus, we felt justified in assuming that the average of the ten standard deviations represented a measure of the variation in gains that might occur in any test group under the conditions of our test. This average standard deviation was found to be 18.1 gm.¹⁵ The variation is essentially that demonstrated in the growth of stock rats of the same age in the same interval of life history (Timson, '32).

THE ELIMINATION OF ANIMALS ESTIMATED AS TOO VARIABLE FOR ASSAY

In our routine assay work, on the basis of a standard deviation of 18.1 gm., we were forced to use large groups of animals and our assays became exceedingly laborious, time-consuming,

¹⁵ The average standard deviation was obtained by the formula $\sqrt{\frac{sd^2}{n}}$ in which sd^2 represents the pooled sum of the squares of the mean differences obtained for each of the ten lots and n represents the degrees of freedom within the lots.

TABLE 1
Summary of forty-nine tests involving 439 rats

PLOT NO.	NUMBER IN LOT	SUPPLE- MENT FED	MILLIGRAMS OF SUPPLE- MENT	'AVERAGE GAINS IN GRAMS IN DIFFERENT TEST PERIODS									VALUES OF \bar{x} ¹		
				1	2	3	4	5	6	7	8	9	10	From the lot	At the 5% level
I	108	Sweet potato	35	39.9	44.4	48.5	50.8	37.4	45.5	41.9	60.7	51.6	45.0	0.3905	0.4090
II	40	Sweet potato	30	40.3	48.7	46.0	52.0	43.5						0.4046	0.4947
III	39	Sweet potato	25	28.2	53.6	30.2	42.7	38.9						0.6431	0.8767
IV	17	Sweet potato	20	13.2	50.2	33.2	46.7	50.2						0.7423	0.8767
V	27	Tomato	175	54.6	40.5	58.2	49.7							0.3128	0.5427
VI	69	Tomato	125	46.8	37.5	28.0	41.0	40.7	46.8	40.1	42.1	37.5		0.3415	0.3702
VII	56	Tomato	100	20.6	28.5	32.2	35.0	28.5						0.0899	0.4947
VIII	22	Carotene	0.001	61.1	47.2									0.5416	0.7386
IX	36	Carotene	0.0005	32.7	34.7									1.0821	2.7588
X	23	Carotene	0.0004	25.0	21.0									0.2920	0.7141

¹ Fisher, '30, section 41.

and expensive. We, therefore, next attacked the problem of reducing the average variability of the gains of our test rats. As all investigators know, no rejection of animals can be made on the basis of gross observations at the end of the depletion period. The only possible line of approach for our study seemed to lie in a determination of possible relationships that might exist between data collected in the depletion and assay periods of the test.

The laboratory records permitted the tabulation of the following items descriptive of the animal in the depletion period: age at weaning, weight at weaning, weight at end of depletion period, the number of days required for depletion, the total gain during depletion, the number of days constant weight was maintained in the critical period, the severity of the xerophthalmia, the gain or loss during the critical period, and the total quantity of food consumed during the depletion period. There were also complete records pertaining to the growth of the animals and the plane of food consumption in the assay period when some source of vitamin A was fed.

These data led us to ask two questions. First, what is the relationship between the quantity of food consumed and rate of growth when vitamin A is added to the deficient diet. Second, what is the relationship between data describing the behavior of the animal in the interval of depletion and the later growth induced by vitamin feeding.

Simple correlation of the factors involved answered the first question, coefficients being determined for all lots of rats represented in this study. No significant relation was found to exist between food intake and concomitant growth, no matter what level of vitamin A was fed.

In regard to the second question, multiple regression showed that of the nine depletion-history factors listed above, six contributed important information about subsequent growth. These are shown in table 2 where all the standard partial regression coefficients (Wallace and Snedecor, '31) are presented. Three were of outstanding importance in determining later gain. For example, the intake of food during the de-

pletion period was directly related to later growth increments whereas the time required for depletion and rate at which the rat grew in this interval bore inverse relationships to the gain made in the actual assay period. Actually, body weight at the end of the depletion period, as shown by simple correlation,

TABLE 2

Multiple correlation between behavior in depletion period and later growth in assay period

Multiple correlation of nine factors ¹ and gain in 8 weeks, 0.394				
Multiple correlation of six factors ¹ and gain in 8 weeks, 0.388				
Multiple correlation of six factors ¹ and gain in 5 weeks, 0.385				

FACTORS	STANDARD PARTIAL REGRESSION COEFFICIENTS SHOWING RELATION OF VARIABLE TO GAIN			DOES VARIABLE CONTRIBUTE IMPORTANT INFORMATION ABOUT GAIN ?
	Nine factors and 8-week test period	Six factors		
		8-week test period	5-week test period	
Age at weaning	— 0.0044	No
Weight at weaning	— 0.1308	— 0.1590	— 0.1723	Yes
Weight at end of depletion	— 0.0660	No
Number of days required for depletion of body stores of vitamin	— 0.3670	— 0.2949	— 0.3852	Yes
Total gain in weight during depletion period	— 0.2536	— 0.3471	— 0.1848	Yes
Critical period				
Number of days of maintenance of constant weight	— 0.1545	— 0.1675	— 0.0880	Yes
Severity of xerophthalmia	+ 0.0668	No
Gain in weight	+ 0.1183	+ 0.1263	— 0.0572	Yes
Weight of total food intake during depletion period	+ 0.3451	+ 0.3217	+ 0.2317	Yes

¹ Those factors contributing important information about gain in test period.

was an important factor but its influence was eliminated in the multiple regression because this weight was the sum of the original weight of the animal and the gain it made in the depletion period.

No information was lost when the items, unimportant insofar as later gain was concerned, were eliminated from the study because multiple correlation coefficients calculated on the basis of nine and six factors, respectively, were found

to be nearly identical, i.e., 0.394 and 0.388. In a group of this size, coefficients as large as these show a highly significant relationship between factors studied.

Since the growth-determining factors were all found in depletion period, expected gain in the assay period was now expressed in terms of a regression equation in which gain in body weight became the dependent variable and the six correlated factors, the independent variables. With the equation established, the response of any animal to vitamin A feeding could be estimated when the data pertaining to its depletion history were used in solving the equation.

The data allowed the calculation of ten different regression equations, each based on the response of a lot to a specific dosage of vitamin A. However, since simple correlation coefficients obtained in a series of preliminary studies showed that the relation between these depletion factors and subsequent growth, were of the same order in each lot, the same relationship could be expected to hold within limits, in a composite group fed various levels of the vitamin. The equation finally obtained based on data pertaining to 577 animals, read as follows:

$$\bar{X} = 107.24 - 0.38A - 1.74B - 0.54C - 3.46D + 1.16E + 0.18F$$

where A = weight in grams at weaning; B = number of days required for depletion of bodily stores of the vitamin; C = total gains in grams during depletion; D = number of days of constant weight at end of depletion period; E = gain or loss in weight in grams during the critical period; F = weight of total food intake in grams during the depletion period; and \bar{X} = estimated gain in grams during the test period.

The value of this equation for the estimation of future growth performance is illustrated by comparing estimated gains with the actual growth records of ten rats selected at random from the group of 577 rats (table 3). Since the equation was based on records of animals fed different levels of vitamin supplement, the actual comparison was not made until the predicted gain of each animal was adjusted to the mean gain of its own specific assay group by subtracting or

adding the difference between the mean gain of the specific group to which a rat belonged and that (34.8 gm.) of the entire lot of 577 rats. Since only one rat failed to behave according to the information furnished by the regression equation, the equation appears to be a valuable tool for predicting at the end of the depletion period whether average or erratic performance can be expected of any individual rat.¹⁶

We next determined the more variable of the animals among the 577 by substitution of data relating to each rat in the regression equation. When the range suggested by the mean

TABLE 3

Gains of individual female rats estimated at end of depletion period by means of the regression equation and the gains actually made in succeeding 8-week period of supplementary feeding

RAT NO.	ESTIMATED GAIN	ACTUAL GAIN
	<i>gm.</i>	<i>gm.</i>
6660	35	35
6676	42	53
6459	40	38
6486	34	30
6653	34	— 11
6668	32	20
6423	37	34
6761	40	45
8861	36	48
9105	34	34
11126	15	6

gain \pm the laboratory standard deviation was used for judging future behavior of an animal, erratic performance was estimated for 20% of the animals. We felt, however, that this range could be justifiably decreased because the inherent nature of the regression equation fixes the range in estimated gains below that of the actual gains. The range was therefore arbitrarily decreased and only those animals were considered suitable for testing whose gains fell within the range of the mean gain \pm 5 gm. Fifty per cent of the 577 animals met this requirement, the standard deviation of their gains in the test period being 16.1 gm.

¹⁶ The calculations involved in the estimation may be simplified in routine work by the construction of an alignment chart (Swett, '28).

Fisher ('37) has described a quantity known as invariance, that measures the information supplied by any particular experiment. In this case, $1/(18.1)^2$ has been taken as one unit of information because 18.1 represents the original standard deviation. After discarding the unsuitable rats, the amount of information yielded by the experiment was greater than before, for with the standard deviation dropping to 16.1, the information supplied equals $\frac{18.1^2}{16.1^2}$, or an increase of 26%.¹⁷

Of importance is the fact that the elimination of non-standard animals did not affect the normality of the distribution of the remaining data as shown by calculations that tested for skewness and kurtosis of the curve, nor did the removal of data pertaining to undesirable animals greatly alter the average gains of the ten lots of rats fed different dosages of vitamin A-carrying supplement. Results are shown in table 4.

However, the variability in every test lot but one (table 4) was decreased by the elimination of unpromising animals. In each of the lots, the standard deviation of the rats retained was smaller than the standard deviation of the original lot, whereas that of the rats eliminated was greater.

The statistic, 16.1, has been designated as the laboratory standard deviation. It becomes, therefore, the measure of the approximate variation that we shall expect to find in any assay conducted in our laboratory in the future under these conditions in an 8-week test period.

SHORTENING THE TEST PERIOD

In studies of gains made by various assay groups, a decrease in the weekly increments in weight often occurred after 35 days of supplementary feeding, especially when A was offered at one of the lower levels. This was associated with increased variability within the lot. Would the reliability of the test be improved in our case by a shortening of the experimental period? Other authors have proposed this step but with one or two exceptions (Coward, '33; Sherman and Burtis, '35), recommendations are not fortified by adequate studies.

¹⁷ $I = \frac{V_1}{V_2}$, in which I represents information and V, the variance or the square of the standard deviation.

We now re-analyzed the data representing the growth induced in the original ten test lots on the basis of responses obtained in the first 5 weeks of the test period only. We

TABLE 4

Mean gain with standard deviation of ten lots of rats, and of the portions of each retained and discarded over test periods, 8 and 5 weeks long

LENGTH OF TEST PERIOD	SUPPLEMENTARY FOOD	ORIGINAL LOTS			GROUPS RETAINED			DISCARDED GROUPS		
		Number of rats	Mean gain	s	Number of rats	Mean gain	s	Number of rats	Mean gain	s
8 weeks	mg.		gm.	gm.		gm.	gm.		gm.	gm.
	Sweet potato									
	35	108	44.7	17.4	56	46.8	16.0	52	42.5	19.0
	30	40	45.6	19.4	21	39.8	18.9	19	52.1	20.4
	25	39	34.0	17.7	19	36.3	15.3	20	31.7	20.8
	20	17	33.1	20.0	10	32.5	16.0	7	41.4	29.6
	Tomato									
	175	27	50.7	13.1	22	52.4	12.5	5	43.6	17.7
	125	69	34.9	17.8	45	36.4	15.6	24	32.0	21.8
	100	56	17.9	20.2	30	22.1	18.4	26	15.6	22.6
	Carotene									
	0.001	22	50.4	19.5	7	48.6	23.3	15	50.7	19.2
	0.0005	36	28.4	16.9	16	34.6	15.7	20	23.6	18.2
	0.0004	23	16.6	17.7	11	18.8	14.0	12	14.7	21.0
5 weeks	Sweet potato									
	35	108	34.2	14.4	65	35.8	11.7	43	31.7	17.7
	50	40	35.4	15.4	19	32.2	14.8	22	38.1	16.2
	25	39	28.5	12.4	19	29.5	10.8	20	27.6	14.0
	20	17	29.8	17.2	9	24.4	14.8	8	35.6	19.2
	Tomato									
	175	27	38.0	7.4	18	37.8	6.5	9	38.2	8.9
	125	69	30.9	11.0	44	31.4	10.9	25	30.0	11.5
	100	56	21.9	17.9	30	24.8	14.6	25	13.6	20.4
	Carotene									
	0.001	22	40.5	13.4	14	41.4	16.0	8	39.0	7.8
	0.0005	36	25.6	11.2	18	29.8	10.4	18	21.4	12.3
	0.0004	23	18.1	15.5	12	23.3	9.2	11	12.4	20.9

found a marked improvement in the assay. The standard deviation was reduced from 18.6 to 13.9. The variations in growth response occurred in large part in the last few weeks of the long experimental period. No significant difference

could be demonstrated between the mean gains made by the animals in the 5- and 8-week periods, except in one case (table 5). Oser's statement ('35) that where steady minimal growth is a criterion the longer the test period the more consistent is the response apparently does not apply in our situation.

Several factors may account for the greater variability in mean increments of growth in the last weeks of the experiment. The variation in the mean weekly rates of growth increases in normal rats of the same approximate age of these assay rats

TABLE 5

Mean gains made by groups of depleted animals fed different sources of vitamin A for two specific intervals of time

SUPPLEMENTARY FOOD	NUMBER OF RATS PER GROUP	TIME SUPPLEMENT WAS FED				MEAN DIFFERENCES IN GAINS IN WEIGHT
		8 weeks		5 weeks		
		Mean gain in weight	s	Mean gain in weight	s	
35 mg. sweet potato	108	gm. 44.7	gm. 17.4	gm. 34.2	gm. 14.4	gm. 10.5
30 mg. sweet potato	40	45.6	19.4	35.4	15.4	10.2
35 mg. sweet potato	39	34.0	17.7	28.5	12.4	5.5
20 mg. sweet potato	17	33.1	20.0	29.8	17.2	3.3
175 mg. tomato	27	50.7	13.1	38.0	7.4	12.7 ¹
125 mg. tomato	69	34.9	17.8	30.9	11.0	4.0
100 mg. tomato	56	17.9	20.2	21.9	17.9	— 4.0
1.0 γ carotene	22	50.4	19.5	40.5	13.4	9.9
0.5 γ carotene	36	28.4	16.9	25.6	11.2	2.8
0.4 γ carotene	23	16.6	17.7	18.1	15.5	— 1.5

¹ This difference only is significant as shown by values of *z*.

(Timson, '32). Also, in the longer test period, the experiment extends beyond the linear portion of the normal growth curve. Finally, it is possible, especially with minimal doses of vitamin A, that inroads of infection do not manifest themselves until the later part of the experiment.

Coward ('33) analyzed growth response at various intervals and concluded that the increase in accuracy obtained by the prolongation of the vitamin A test beyond a period of 3 weeks was too slight to justify the extra expenditure and labor involved. Whether the test period can be shortened to less than 5 weeks in our laboratory as recommended by the U. S.

Pharmacopoeia Committee ('34) without sacrifice of accuracy will be tested later. The similarity in the standard deviation of the total gains at weekly intervals obtained in two different laboratories should be noted at this point. Coward ('33) found the standard deviation of the increase in weight to be 5.6, 8.1, 9.8, 11.1 and 11.9 gm. in succeeding weeks, whereas in one lot of our animals the standard deviations for 8 successive weeks were 3.8, 6.9, 6.8, 8.8, 10.9, 12.3, 14.5 and 17.8 gm.

To test the possibility of reducing the variability of the 5-week assay by the elimination of unsuitable animals, a new regression equation was calculated, i.e.,

$$\bar{X} = 76.88 - 0.28A - 1.31B - 0.23C - 1.23D - 0.36E + 0.09F$$

The data pertaining to each rat were substituted in the equation as before and those whose estimated gain varied from the mean (29.3 gm.) by more than ± 4 gm. were eliminated. The standard deviation dropped to 12.0 gm. The information now contributed by the experiment is $\frac{(18.1)^2}{(12.0)^2} = 2.27$ units in contrast to 1 unit in the original experiment, an increase of 127%. This means that in comparing test groups composed of fifteen to twenty animals, differences in mean gains of 7 to 8 gm. can be considered significant.¹⁸ In the original assay, a difference of 11 gm. in the results of two assays being compared was necessary in order to make this interpretation. The figure, 12.0 becomes our laboratory standard deviation for a 5-week test period when the less desirable animals are eliminated.

PRACTICAL APPLICATION OF RECOMMENDED PROCEDURES

The studies herein presented show that a judicious handling of experimental data may markedly increase the uniformity and thence the reliability of an assay. We recommend first, the inclusion in the final assay of only a predetermined portion

¹⁸ The following formula was used, $s_{MD} = \sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}}$ where s_{MD} represents the average deviation of the mean difference in gains made by two test lots; s , the laboratory standard deviation; and n , the number of animals in each lot. The minimum mean difference for significance is $2s_{MD}$.

of the depleted rats. The elimination of rats of irregular behavior as estimated by means of the regression equation in no way affects the normality of the distribution of remaining data. Second, evidence has been presented that shows that the use of a 5-week test period is superior to one 8 weeks long. As a result, the standard deviation may be reduced from 18.1 gm. to 12.0 gm. with increase in the information supplied by the experiment of 127%. However, the usefulness and efficacy of technics of this kind must be fully tested. Several comparisons and tests were therefore made.

A comparison of the reliability of our assay with those of other investigators is interesting. As far as we know, Coward ('32 and '33) is the only other worker who has subjected as large a mass of data as ours to similar analysis. She found the average standard deviation of the gains of 672 females in an experimental period of 5 weeks to be 11.0 gm.; in a later test involving 1110 rats, 11.9 gm. Her animals are, therefore, less variable than are our unselected rats (standard deviation, 13.9). However, by selection, the variation in response of our rats can be reduced to 12.0 gm., showing that under these circumstances, assays from the two laboratories are more directly comparable than they were in the first instance.

The use of the regression equation as a measure for the identification of irregular animals also needed justification from the standpoint of results obtained in the analysis of data not represented in the regression equation. If the equation furnishes a reliable basis for the elimination of unsuitable animals, one-half of the animals in a new assay group should be found sub-standard at the end of the depletion period and the standard deviation from the mean gain made by the remaining animals in a 5-week assay period should approximate the laboratory standard deviation, i.e., 12.0 gm.

In order to test the hypothesis, a group of twenty-eight animals were depleted of vitamin A in the standard routine manner 1 year after the equation was calculated. Upon substitution of individual data (table 6) in the regression equation, erratic response was predicted of fifteen rats, the esti-

TABLE 6

Example of reduction of size of an experimental lot at the end of the depletion period on the basis of a suitable regression equation

Regression equation: $\bar{X} = 76.88 - 0.28A - 1.31B - 0.23C - 1.23D - 0.36E + 0.09F$ (5 weeks)									
GROUPS FORMED	RAT NO.	A WEIGHT AT WEANING	B LENGTH OF DEPLETION PERIOD	C GAIN IN WEIGHT IN DEPLETION PERIOD	D TIME OF CONSTANT WEIGHT OF DEPLETION PERIOD	E GAIN OR LOSS IN WEIGHT IN CRITICAL PERIOD	F FOOD CONSUMED IN DEPLETION PERIOD	\bar{X} ESTIMATED GAIN IN TEST PERIOD	X ACTUAL GAIN IN TEST PERIOD
		gm.	days	gm.	days	gm.	gm.	gm.	gm.
Animals eliminated	13777	44	28	54	5	- 2	152	23.7	
	13807	42	30	50	5	2	175	23.2	
	13886	40	28	63	5	3	197	25.0	
	14041	41	30	71	5	2	215	22.2	
	13466	38	32	62	5	1	187	20.4	
	13474	39	26	61	5	0	149	25.1	
	14520	35	14	17	5	2	58	43.2	
	13908	46	17	14	5	3	96	39.9	
	14124	50	28	62	5	2	194	22.5	
	11338	48	36	92	4	2	267	13.5	
	14039	36	29	76	5	4	214	23.0	
	13783	44	29	60	4	1	169	22.7	
	13862	40	32	66	5	3	204	19.7	
Animals retained	13836	36	29	62	6	1	179	22.9	
	13808	42	30	53	5	0	178	23.5	
	13801	30	23	50	6	4	131	29.8	21
	13645	44	21	49	5	3	131	30.3	31
	13722	46	22	53	5	2	129	27.7	46
	13747	36	22	42	5	2	114	31.7	42
	13955	40	25	56	5	4	147	25.7	38
	13909	44	24	41	4	- 4	139	32.7	29
	14099	42	22	62	5	4	148	27.7	31
	14066	37	27	58	5	3	171	26.0	26
	14064	36	25	66	5	2	171	27.4	15
	14047	31	27	53	5	2	175	29.5	18
	14046	44	29	59	3	1	193	26.3	17
13482	42	25	54	5	4	162	26.9	12	
13473	41	24	51	4	- 1	119	28.4	32	

mated response of all of these falling outside the range, 29.3 ± 4 gm.

The thirteen remaining rats were fed 0.0006 mg. of carotene in the test period. The mean gain of this group was 27.5 gm., with a standard deviation of 10.5 gm. The agreement of the resulting standard deviation with that discussed above was gratifying. This test also suggested that our stock colony did not represent a shifting population. Otherwise, the laboratory standard deviation could not be used for estimating the reliability of a test.

We wondered also whether undesirable animals could be identified in tests run before the assay was as highly standardized as it is at present. An assay conducted in 1930 in which rats received 30 mg. of butterfat as the sole source of vitamin A was therefore re-analyzed. The mean gain of the original group over an experimental period of 5 weeks was 18.7 gm. with a standard deviation of 17.1 gm. In scanning the data pertaining to this test (table 7), casual inspection shows in the light of our present criteria, that at least twelve animals were over-depleted. Upon testing with the regression equation, these rats were in the group estimated as erratic. The mean gain made by the irregular animals was 13.6 gm., that of the remaining group, 30.4 gm. The standard deviations were 17.7 gm. and 8.9 gm., respectively. Probably 30.4 gm. more nearly represents the true response of depleted rats to the daily administration of this quantity of butterfat than does the result of the original analysis, and is one less colored by pathologic influences.

The recommended procedures also are of direct practical importance. Estimations showed that both the time involved for the completion of an assay and the number of animals needed for the actual test may be lowered in the neighborhood of 40%. Laboratory expense is also cut a corresponding degree.

If an investigator does not feel justified in actually eliminating animals at the end of the depletion period and in shortening the test period as suggested herein, he may try the effect for himself without disturbing his usual procedure.

TABLE 7
Estimated and actual gains made by desirable and undesirable animals in a lot composed of twenty-three rats, in the main, over-depleted

Regression equation: $\bar{X} = 76.88 - 0.28A - 1.31B - 0.23C - 1.23D - 0.36E + 0.09F$ (5 weeks)									
GROUPS FORMED	RAT NO.	A WEIGHT AT WEANING	B LENGTH OF DEPLETION PERIOD	C GAIN IN WEIGHT IN DEPLETION PERIOD	D TIME OF CONSTANT WEIGHT OF DEPLETION PERIOD	E GAIN OR LOSS IN WEIGHT IN DEPLETION PERIOD	F FOOD CONSUMED IN DEPLETION PERIOD	\bar{X} ESTIMATED GAIN IN TEST PERIOD	X ACTUAL GAIN IN TEST PERIOD
Undesirable animals	6153	gm. 42	days 22	gm. 48	days 4	gm. - 2	gm. 145	gm. 34.1	gm. 33
	6164	50	29	62	7	2	221	21.2	15
	6225	60	30	47	12	3	209	12.9	24
	6322	52	21	49	5	- 5	171	34.6	19
	6205	40	31	54	9	4	190	17.2	11
	6239	40	33	74	8	4	233	15.1	14
	6169	55	32	77	9	5	247	11.2	27
	6213	51	25	49	7	3	166	23.8	17
	6262	54	35	63	8	4	299	10.7	- 15
	6220	47	32	60	11	4	209	11.8	- 4
	6163	48	33	77	5	5	218	14.2	17
	6155	46	36	78	9	8	263	10.1	- 24
	6238	45	28	59	6	0	181	22.9	36
	6224	56	28	54	7	3	191	19.6	- 2
	6156	40	21	44	3	- 3	129	37.1	40
	6211	44	26	78	7	0	180	20.2	9
	6319	58	21	45	5	1	158	30.5	28
	6329	45	21	52	5	2	144	30.9	32
	6327	52	21	47	5	0	150	31.3	33
	6321	55	21	43	5	- 2	158	32.8	39
	6161	47	25	67	3	3	198	28.6	27
	6154	42	22	59	4	- 1	153	31.9	36
	6221	45	25	48	6	3	167	27.1	18
Desirable animals									

Let him compute the expected gains of his animals, designating in advance the half of the animals expected to make the least erratic gains. He can then compute results on both the half and the entire lot, thus learning if his results compare with ours, but without risk of sacrificing his test.

SUMMARY

In a study of methods for the improvement of the bio-assay for vitamin A, the average standard deviation from the mean gain has been chosen as the criterion in making comparisons.

The possibility of improving the bio-assay for vitamin A by strict laboratory standardization of the generally accepted method has been demonstrated.

Further reduction of the variability of the assay has been effected through two channels. First, animals were eliminated from the assay group that gave indications in the depletion period of erratic response to the feeding of the test substance. These eliminations were made on the basis of estimations of future gain by means of a regression equation calculated on certain data collected in the depletion period and later gain in the test period. Second, the uniformity and thence the reliability of the assay has been increased by shortening the test period from 8 to 5 weeks.

The adoption of the two procedures in conducting the assay increased the information yielded by the test 127%.

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THE INFLUENCE OF DIET ON THE NITROGEN BALANCES OF PRE-SCHOOL CHILDREN¹

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TWO FIGURES

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Although there has been much discussion in the literature concerning the value of high and low protein diets, the protein needs of pre-school children are still questioned. Experimental evidence suggests that children might develop faster and be physically stronger if they had a high rather than a low protein diet. These advantages may arise not only from an increase in protein content, but also from a proper balance between the protein and the other constituents in the diet or between the various essential amino acids. At the present time, it is impossible to prepare an adequate synthetic diet for children from different amino acids. Thus the value of protein must be determined by varying its amount and not by varying the amounts of different amino acids in the diet. In previous studies, the experimental periods were exceedingly short or there were several variables beside the amount of protein in the diet. Therefore, the purposes of the present study were, first, to determine the variations in the nitrogen balances of the pre-school children who received a constant diet and, second, to find the changes in the nitrogen balances when only the protein content of the diet was changed.

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Previous investigators do not agree as to the exact amount of protein needed. Sherman ('37) says that the growing child should have 10% of the total caloric intake in the form of protein or more than twice as much per unit of weight as the adult. According to Rose ('33) this amount would be approximately 3 gm. per kilogram. Since the caloric intake of a child varies in relation to his activity, a standard based on percentage of calories eaten may be quite variable. Therefore, the question arises as to the exact amount necessary for optimum nutrition. Several investigators have determined the minimum quantity necessary. Parsons ('30) says that normal children from 4 to 8 years of age can maintain a positive nitrogen balance on 0.5 to 1.1 gm. of protein per kilogram, if the caloric intake is adequate. Bartlett ('26) found that diabetic children needed practically the same amount, 0.6 to 1.0 gm. per kilogram, while Boyd ('25) could not obtain positive nitrogen balances on less than 1.25 gm. of protein per kilogram. Although these subjects on low nitrogen intakes did gain weight, in the light of the studies which Wang, Hawks and Hayes ('28), Wang, Hawks and Kaucher ('28), Wang, Kern and Kaucher ('29) made on undernourished children, their nutritional development may not have been optimal. These authors ('29) noted positive nitrogen balances on low levels of nitrogen intake, but higher retentions and greater gains in weight following an increase in protein content of the diet. Daniels and her co-workers ('35) in their study on the protein needs of pre-school children, found that, based on creatinine elimination in terms of theoretical weight, the children retained the greatest amount of nitrogen when they received approximately 3.2 gm. of good quality protein per kilogram. Although these studies have added valuable information to our knowledge of protein metabolism, all of the factors which influence the utilization are not known.

The results of the present study may give further information because the period of study was fairly long and there were few variations in the procedure (Hawks, Bray and Dye, '37 a). Six normal pre-school children received two adequate

diets, one containing 3 and the other 4 gm. of protein per kilogram of body weight. The first diet continued for 21 days and the second for 15 or 24 days. Although the authors took every precaution to have a constant diet, there were slight fluctuations not only from period to period but also within a period. In the first experiment on two children, the calories were constant but the protein was not the only variable because skimmed milk, egg and beef raised the protein content. In the second experiment on four children, there were fewer variables, for egg white and gelatin increased the protein and the omission of butter kept the calories at the same level. These changes in diet increased the amount of protein from animal sources 8.5 and 6.8% in the two experiments, respectively. Although Belousov and Gilman ('34) found that children utilized animal protein better than vegetable protein, the differences in the present study were probably too slight to influence the results. Some investigators (Edelstein, Langer and Langstein, '31; Edelstein, '32, and Funnell and her associates, '36) found that an increase in bulk content of the diet decreased protein utilization, while Schultz, Morse and Oldham ('33) said that small increases in bulk had little influence. However, in this experiment, the bulk content could not have been an important factor because there was a difference of only 12.6 gm. of vegetable between the two diets in the first experiment and none in the second. According to a number of investigators (Weber, '32; Krause, '33; Davis, '35, and Röse, '35) who studied the effect of acid and basic diets on the nitrogen retention of infants and children, protein was most effectively utilized when the diet residue was basic. All of the diets in the present study were basic, the medium and high protein diets varying only 8.4 and 4.1 cc. of normal solution in the first and second experiments, respectively. Therefore, the acid base relationship was probably not an influential factor. The change in the calcium and phosphorus content of the diet was negligible in the second experiment, but, in the first, the calcium increased 0.168 gm. and the phosphorus 0.313 gm. Protein, therefore,

TABLE 1
Nitrogen balance data on a constant medium protein diet, expressed per kilogram of body weight

CHILD	EXPERIMENT	PERIOD	INTAKE			OUTPUT				Total		ABSORPTION		RETENTION	
			mg	Dried weight	Nitrogen	Urine		Feces		Total nitrogen	Proportion intake	Total nitrogen	Proportion intake	Total nitrogen	Proportion intake
						mg	%	mg	%						
B	I	1	506	16.9	389	76.8	15.5	4.63	1.31	60	92.3	428	84.5	39	7.7
		2	504	17.1	379	74.6	20.4	6.02	1.42	73	95.0	401	79.6	25	5.0
		3	526	17.2	402	76.4	13.3	4.08	1.17	60	89.7	456	86.7	54	10.3
		4	533	17.4	418	78.4	11.8	3.63	1.10	57	90.2	470	88.2	52	9.8
		5	500	17.3	405	80.9	12.6	3.67	1.18	53	93.5	437	87.4	32	6.5
		6	518	16.8	416	80.4	12.2	3.75	1.13	56	92.6	455	87.8	39	7.4
		7	507	16.9	412	81.4	12.4	3.71	1.10	57	93.8	444	87.6	31	6.2
		Mean	513	17.1	403	78.4	14.0	4.21	1.20	60	92.4	441	86.0	38	7.6
D	I	1	501	16.7	412	82.4	12.8	3.83	1.30	49	95.2	437	87.2	25	4.8
		2	500	16.9	419	83.8	12.0	3.54	1.21	50	95.8	440	88.0	21	4.2
		3	521	17.0	436	83.7	8.8	2.71	0.97	47	92.5	475	91.2	39	7.5
		4	526	17.1	434	82.5	9.3	2.84	1.03	48	91.8	477	90.7	43	8.2
		5	492	17.0	425	86.3	9.8	2.83	1.21	40	96.1	444	90.2	19	3.9
		6	513	16.7	444	86.6	9.9	3.04	1.05	49	96.5	462	90.1	18	3.5
		7	502	16.8	421	83.9	10.4	3.12	1.11	48	94.3	449	89.6	29	5.7
		Mean	508	16.9	427	84.2	10.4	3.13	1.13	47	94.6	455	89.0	28	5.4

V	II	1	439	18.0	393	89.5	33	7.5	1.83	0.78	42	426	97.0	406	92.5	13	3.0
		2	454	17.4	396	87.2	32	7.1	1.84	0.79	41	428	94.3	422	92.9	26	5.7
		3
		4
		5	454	17.6	395	87.0	32	7.1	1.84	0.83	39	427	94.1	422	92.9	27	5.9
		6	466	17.8	402	86.4	33	7.1	1.87	0.80	41	435	93.5	433	92.9	31	6.5
		7	436	17.7	374	85.9	35	7.9	1.95	0.77	45	409	93.8	401	92.1	27	6.2
		Mean	450	17.7	392	87.2	33	7.3	1.87	0.79	42	425	94.5	417	92.7	25	5.5
		1	456	18.7	398	87.5	44	9.6	2.35	0.91	48	442	97.1	412	90.4	14	2.9
		2	473	18.1	398	84.2	46	9.7	2.54	0.92	50	444	93.9	427	90.3	29	6.1
C	II	3	488	18.5	396	81.2	45	9.2	2.44	0.90	50	441	90.4	443	90.8	47	9.6
		4	482	18.5	403	83.6	45	9.4	2.45	0.92	49	448	93.0	437	90.6	34	7.0
		5	471	18.3	397	84.3	45	9.6	2.48	0.94	48	442	93.9	426	90.4	29	6.1
		6	484	18.4	400	82.6	46	9.6	2.51	0.91	51	446	92.2	438	90.4	38	7.8
		7	452	18.3	379	83.9	45	10.0	2.48	0.89	51	424	93.9	407	90.0	28	6.1
		Mean	472	18.4	396	83.9	45	9.6	2.46	0.91	49	441	93.5	427	90.4	31	6.5
		1	427	17.5	356	83.4	52	12.1	2.95	0.92	57	408	95.5	375	87.9	19	4.5
		2	441	16.9	363	82.2	54	12.2	3.18	0.98	55	417	94.4	387	87.8	24	5.6
		3	456	17.3	365	80.1	57	12.4	3.28	1.01	56	422	92.5	399	87.6	34	7.5
		4	453	17.4	363	80.1	59	13.0	3.40	1.07	55	422	93.1	394	87.0	31	6.9
J	II	5	443	17.2	374	84.7	59	13.0	3.44	1.07	55	433	92.7	384	87.0	10	2.3
		6	454	17.3	367	80.8	54	11.9	3.13	0.99	55	421	97.7	400	88.1	33	7.3
		7	423	17.1	342	80.8	54	12.8	3.15	0.99	55	396	93.6	369	87.2	27	6.4
		Mean	442	17.2	361	81.7	56	12.5	3.22	1.00	56	417	94.2	386	87.5	25	5.8

was the chief variable in the two similar experiments, since the percentage of protein from animal sources, the bulk, the acid-base relationship, calcium, phosphorus and calories were practically constant.

EXPERIMENTAL RESULTS

Medium protein diet. The reactions of each child to the standardized medium protein diet was remarkably constant from period to period, but there were some variations in the reactions of different children. Table 1 and figure 1 show

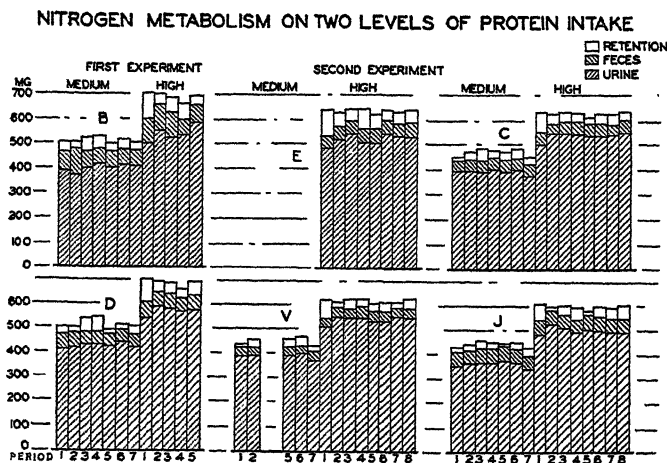


Figure 1

the nitrogen balances for the children who received this diet and table 2 gives the statistical evaluation of the data.

For each child, the total nitrogen output as well as the figures for urine and feces varied little from period to period. The average percentage of the intake excreted both in the urine and feces varied between the children, but the values seemed to compensate each other so that the total excretion represented between 92.4 and 94.6% of the intake. Considering the data for experiments one and two rather than for individual children, Hawks, Bray and Dye ('37 b) previously reported that the variations in diet nitrogen influenced urinary

excretion because the coefficients of variation were similar. Table 2 shows that the coefficients of variation for total output were also practically the same as those for diet and that, in the second experiment, the correlation with intake was high. Feces values apparently had no relation to intake variation and the average figures were quite different for individual children. Therefore, the practically constant diet nitrogen must have caused the children to excrete the same proportion

TABLE 2
Statistical evaluation of nitrogen data on 3-gm. protein diet

	EXPERIMENT	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION WITH INTAKE	CORRELATION WITH DRY WEIGHT OF FOOD	CORRELATION WITH DRY WEIGHT OF FECES
Intake	I	mg. 511 \pm 1.2	mg. 12.0	2.3
	II	457 \pm 1.5	17.2	3.8
Output Urine	I	415 \pm 2.0	19.5	4.7	0.21
	II	384 \pm 1.6	18.6	4.8	0.67
Feces	I	62 \pm 2.7	14.8	23.7	- 0.10	0.15	0.75
	II	46 \pm 1.4	9.3	20.2	- 0.15	- 0.35	0.91
Total	I	477 \pm 1.3	7.0	1.5	0.36
	II	428 \pm 2.2	14.3	3.3	0.84
Absorption	I	448 \pm 3.6	20.2	4.5	0.64
	II	410 \pm 3.4	21.9	5.4	0.86
Retention	I	33 \pm 2.1	11.5	34.6	0.77
	II	27 \pm 1.4	8.9	32.4	0.63

of the intake, but some other factors must have produced the differences in the distribution between the urine and feces. Although the bulk and other diet factors remained constant, the character of the stools varied. Subjects D., B. and J. had rather loose stools while V. had quite constipated ones. Consequently, the dry weight of the stools (table 1) varied between different children but showed high correlations with the feces nitrogen, 0.75 and 0.91 (table 2). Although these subjects did not receive a low nitrogen diet, the results indicated, as did those of Heupke ('34) and Heupke and Belz ('35), that

fecal nitrogen was dependent upon the weight of the feces and that there may have been a constant nitrogen fraction excreted. Schneider ('35), however, noted that with human subjects there seemed to be no constant fraction for metabolic nitrogen, all of it varying in proportion to the intake of dry food. The ratio of fecal nitrogen to dry matter consumed (table 1) showed that this may have been true for some individual children, but, the average ratios for the different children varied according to the nature of the stools, and there was no correlation between the two factors. The ratios were within the range of those which Mitchell ('26) reported for human subjects receiving low nitrogen diets, but they were lower than those which Schneider reported, possibly because the bulk content of the present diet must have been lower than that which he used. Nevertheless, the data indicate that there were individual variations in fecal nitrogen excretion which were not shown in the total excretion.

Since the feces values varied for individual children, the absorption or coefficient of digestibility of the same food varied from 86.0 to 92.7% of the intake. These values were quite constant for each individual child and they showed a high degree of correlation with intake, especially in the second experiment (table 2). The small variations in intake were also reflected in the retentions of these children who were in nitrogen equilibrium. All of the retentions were positive and, considering the number of biological and technical errors, they were remarkably constant, the average values representing from 5.4 to 7.6% of the intake. In spite of the high coefficients of variation in the retention values, there was a definite correlation with intake, 0.77 and 0.63 for the two experiments. Therefore, the data on the medium protein diet show that the children reacted in a similar manner and retained nitrogen in proportion to the period by period variations in diet, even though they did not excrete the same proportion of nitrogen in urine and feces.

High protein diet. The reactions of the children to the high protein diet were similar to those on the medium protein diet.

Yet there were some differences in the amounts and in the proportions excreted both by way of the urine and feces. Figure 1 as well as table 3 show that the total output was higher for each child on every period. During the first period following the change in diet, there was a definite lag in the urinary nitrogen excretion. The values were above those on the medium protein diet but lower than any of the other high protein figures. Since the fecal nitrogen values were not proportionally higher during the first period, the total excretions were low and the retentions high. Although the values during the second and third periods were similar to all others, a preliminary period of 9 days, as used for the data on the urinary nitrogenous constituents (Hawks, Bray and Dye, '37 b), certainly covered the fluctuations due to change in diet. These preliminary values were less constant than those for the medium protein diet or for all of the high protein diet because the statistical evaluation of the data (table 4) showed higher standard deviations and coefficients of variability, and less correlation between intake and the several functions.

In the first experiment, there were only two periods following the preliminary period. Therefore, the differences in the metabolism of the children on the two diets can best be determined by comparing the data for the last five high protein periods in the second experiment with the data for the same children on the medium protein diet. A comparison of these data after the children had reached equilibrium on the two levels of protein intake indicated that there were slight differences in nitrogen utilization (table 5). The percentage of the intake excreted in the urine was higher on the high protein diet, while that excreted in the feces was lower, but again these two values seemed to compensate each other so that the total excretion represented approximately the same constant percentage of the intake that it had on the medium protein diet, from 90.1 to 93.0%. As before, the fecal nitrogen seemed to vary according to the character of the stools, but the nitrogen increase was less than the increase in diet nitrogen and

TABLE 3
Nitrogen balances of children who received a constant diet containing 4 gm. of protein per kilogram

CHILD	EXPERI- MENT	PERIOD	INTAKE		Urine		OUTPUT						ABSORPTION		RETENTION	
			Nitro- gen	Dried weight	Total nitro- gen	Per cent intake gen	Feces				Total		Total nitro- gen	Per cent intake gen	Total nitro- gen	Per cent intake gen
							Total nitro- gen	Per cent intake	Nitrogen per gram dried food	Dried weight	Nitrogen per gram dry feces	Total nitro- gen				
B	I	1	mg	gm.	mg.	%	gm.	gm.	mg.	gm.	mg.	%	mg.	%	mg.	%
		707	17.7	503	71.2	14.3	5.72	1.54	66	604	85.5	606	85.7	103	14.5	
		2	704	17.3	558	79.3	14.9	6.06	1.48	71	663	94.2	599	85.1	41	5.8
		3	691	18.5	529	76.5	15.1	5.63	1.44	72	633	91.6	587	84.9	58	8.4
		4	668	18.1	540	80.9	8.8	3.24	1.11	53	599	89.7	609	91.2	69	10.3
	Mean	5	696	16.0	587	84.3	10.5	4.58	1.30	56	660	94.8	623	89.5	36	5.2
		1-3	701	17.9	530	75.6	14.8	5.80	1.49	69	633	90.4	598	85.2	68	9.6
	Mean	All	693	17.5	543	78.4	12.8	5.05	1.37	64	631	91.2	605	87.2	62	8.8
		D	1	699	17.5	542	77.6	9.5	3.80	1.23	55	609	87.1	632	90.5	90
	2		696	17.2	589	84.7	9.8	3.60	1.17	53	651	93.6	634	91.1	45	6.4
3	687		18.4	581	84.6	9.5	3.55	1.21	54	646	94.1	622	90.5	41	5.9	
4	662		17.9	570	86.0	8.6	3.18	1.09	52	627	94.6	605	91.4	35	5.4	
5	690		15.8	576	83.4	9.0	3.91	0.88	71	638	92.4	628	91.0	52	7.6	
E	Mean	1-3	694	17.7	571	82.3	9.3	3.65	1.20	54	636	91.6	629	90.7	58	8.4
		Mean	687	17.4	572	83.2	9.1	3.61	1.11	57	635	92.3	624	90.9	52	7.7
	II	1	645	18.8	488	75.7	8.1	2.78	1.00	52	540	83.8	593	91.9	105	16.2
		2	637	18.8	524	82.4	8.5	2.88	1.00	55	579	90.9	582	91.5	58	9.1
		3	648	18.7	551	85.1	8.1	2.82	1.04	51	604	93.2	595	91.9	44	6.8
		4	646	19.1	516	79.9	8.7	2.95	1.06	54	573	88.6	589	91.3	73	11.4
		5	625	19.0	514	82.2	9.6	3.14	1.12	54	574	91.8	565	90.4	51	8.2
	Mean	6	642	19.3	548	85.3	9.2	3.08	1.09	54	607	94.5	583	90.8	35	5.5
		7	638	17.6	541	84.8	8.5	3.10	1.07	51	595	93.3	584	91.5	43	6.7
		8	649	18.9	538	82.8	9.1	3.15	1.05	56	597	91.9	590	90.0	52	8.1
Mean	1-3	643	18.8	521	81.1	8.2	2.83	1.01	53	574	89.3	590	91.8	69	10.7	
	Mean	640	18.8	531	83.0	9.0	3.08	1.08	54	589	92.0	582	91.0	51	8.0	
	Mean	641	18.8	528	82.3	8.7	2.99	1.05	53	584	91.0	585	91.3	57	9.0	

V	II	1	622	18.1	508	81.5	36	5.9	2.01	0.80	45	544	87.4	586	94.1	78	12.6
		2	614	18.2	551	89.7	40	6.6	2.22	0.89	45	591	96.3	574	93.4	23	3.7
		3	625	18.0	546	87.4	39	6.2	2.16	0.88	44	585	93.6	586	93.8	40	6.4
		4	623	18.5	548	87.9	48	7.7	2.61	0.99	49	596	95.6	575	92.3	27	4.4
		5	603	18.3	528	87.5	47	7.8	2.59	0.91	52	575	95.3	556	92.2	28	4.7
		6	616	18.5	533	86.5	41	6.7	2.23	0.86	48	574	93.2	575	93.3	42	6.8
		7	616	17.0	552	89.6	39	6.3	2.30	0.84	46	591	95.9	577	93.7	25	4.1
		8	627	18.2	549	87.6	40	6.3	2.18	0.82	49	589	93.9	587	93.7	38	6.1
		Mean	1-3	620	18.1	535	86.2	38	6.2	2.13	0.86	44	573	92.4	93.8	47	7.6
		Mean	4-8	617	18.1	542	87.8	43	7.0	2.38	0.88	49	585	94.8	93.0	32	5.2
G	II	Mean	All	618	18.1	539	87.2	41	6.7	2.29	0.87	47	580	93.9	93.3	38	6.1
		1	643	18.8	503	78.3	56	8.7	2.98	0.99	57	559	87.0	587	91.3	84	13.0
		2	634	18.7	551	86.9	41	6.5	2.20	0.83	49	592	93.4	593	93.5	42	6.6
		3	641	18.5	553	86.2	47	7.4	2.56	0.99	48	600	93.6	594	92.6	41	6.4
		4	638	18.9	548	85.9	52	8.2	2.77	1.05	49	600	94.1	586	91.8	38	5.9
		5	619	18.8	641	87.3	51	8.2	2.71	0.98	52	592	95.5	568	91.8	27	4.5
		6	634	19.0	541	85.3	51	8.0	2.67	0.98	52	592	93.3	583	92.0	42	6.7
		7	629	17.4	544	86.5	45	7.2	2.61	0.90	50	589	93.7	584	92.8	40	6.3
		8	641	18.6	558	87.0	49	7.7	2.65	0.89	55	607	94.7	592	92.3	34	5.3
		Mean	1-3	639	18.7	536	83.8	48	7.5	2.53	0.94	51	584	91.3	92.5	55	8.7
J	II	Mean	4-8	632	18.5	546	86.3	50	7.9	2.68	0.96	52	596	94.2	92.1	36	5.8
		Mean	All	635	18.6	542	85.4	49	7.7	2.64	0.95	52	591	93.1	92.3	44	6.9
		1	605	17.6	480	79.4	61	10.1	3.48	1.02	60	541	89.5	544	89.9	64	10.5
		2	596	17.6	523	87.8	57	9.6	3.26	1.00	57	580	97.4	539	90.4	16	2.6
		3	599	17.3	506	84.3	59	9.9	3.43	1.01	58	565	94.2	540	90.1	34	5.8
		4	595	17.6	488	82.1	56	9.3	3.15	0.96	58	544	91.4	539	90.7	51	8.6
		5	579	17.6	500	86.3	61	10.5	3.45	1.02	60	561	96.8	518	89.5	18	3.2
		6	593	17.8	497	83.7	59	10.0	3.33	1.04	57	556	93.7	534	90.0	37	6.3
		7	590	16.2	491	83.2	59	10.0	3.65	1.04	57	550	93.2	531	90.0	40	6.8
		8	600	17.4	489	81.5	59	9.9	3.41	1.04	57	548	91.4	541	90.1	52	8.6
	Mean	1-3	600	17.5	503	83.8	59	9.9	3.39	1.01	58	562	93.7	541	90.1	38	6.3
		4-8	591	17.3	493	83.4	59	9.9	3.40	1.02	58	552	93.3	532	90.1	39	6.7
		All	595	17.4	497	83.5	59	9.9	3.40	1.02	58	556	93.4	536	90.1	39	6.6

TABLE 4
Statistical evaluation of nitrogen data on 4-gm. protein diet

	PERI- EX- MENT	PERIOD	MEAN	STAND- ARD -DEVI- ATION	COEF- FICIENT OF VARI- ATION	CORRE- LATION WITH INTAKE	CORRELA- TION WITH DRY WEIGHT OF FOOD	CORRELA- TION WITH DRY WEIGHT OF FECES
			mg.	mg.				
Intake	I	All	691 \pm 1.7	13.7	2.0
		1-3	697 \pm 1.1	7.1	1.0
	II	All	622 \pm 1.4	19.9	3.2
		1-3	626 \pm 2.3	18.5	3.0
		4-8	620 \pm 1.8	20.5	3.3
Output Urine	I	All	558 \pm 4.1	32.7	5.9	- 0.21
		1-3	551 \pm 5.7	36.0	6.5	0.43
	II	All	527 \pm 1.9	27.2	5.2	0.53
		1-3	524 \pm 3.9	31.3	6.0	0.32
		4-8	528 \pm 2.2	25.1	4.8	0.68
Feces	I	All	76 \pm 4.2	19.7	26.1	0.56	0.18	0.79
		1-3	84 \pm 5.7	21.3	26.3	0.39
	II	All	51 \pm 0.9	7.7	15.1	- 0.20	- 0.04	0.86
		1-3	50 \pm 1.7	8.7	17.5	- 0.18
		4-8	52 \pm 1.1	7.1	13.7	- 0.17	0.05	0.85
Total	I	All	633 \pm 4.9	23.0	3.6	0.26
		1-3	634 \pm 6.5	23.7	3.7	- 0.27
	II	All	578 \pm 2.5	21.0	3.6	0.53
		1-3	573 \pm 4.5	23.0	4.0	0.20
		4-8	581 \pm 3.0	19.9	3.4	0.77
Absorption	I	All	615 \pm 3.3	15.7	2.6	0.13
		1-3	613 \pm 5.2	19.0	3.1	0.10
	II	All	571 \pm 2.7	22.6	4.0	0.91
		1-3	576 \pm 4.3	22.0	3.8	0.85
		4-8	578 \pm 3.5	23.0	4.1	0.91
Retention	I	All	57 \pm 5.0	23.5	41.2	0.31
		1-3	63 \pm 7.4	27.0	42.9	0.46
	II	All	44 \pm 2.3	19.4	43.8	0.41
		1-3	52 \pm 5.1	26.2	50.1	0.47
		4-8	40 \pm 1.9	12.4	31.1	0.34
Retention 3 children	II	All	40 \pm 2.3	16.7	41.6	0.29
		1-3	47 \pm 5.3	23.5	50.2	0.43
		4-8	36 \pm 1.7	9.5	26.5	0.58

practically the same as the increase in dry weight of the feces. Therefore, the nitrogen per gram of dried feces decreased, and there was still a correlation of 0.85 between the two sets of data. Although the only differences in food were the addition of egg white and gelatin and the omission of butter, the dry weight of the food increased slightly, but not in proportion to the fecal nitrogen increase because the fecal nitrogen per gram of dried food was higher and there was still no correlation between these two factors. Thus these data seem to indicate that there must have been a constant nitrogen fraction excreted in the feces.

The high protein diet increased the coefficient of digestibility or the percentage of the intake absorbed as much as 2.6%, but caused less than 1.0% difference in the amount retained (table 5). The increase in absorption as well as the differences in fecal nitrogen may have been due to the fact that gelatin and egg white had higher biological values than the other proteins in the diet. It was not due to an increase in the variability in the data for the coefficient of variation was no higher. Fluctuation in diet nitrogen apparently caused the variations in absorption because the correlation between the two factors was 0.91. Since the retention values represented a proportion of the intake similar to that on the medium protein diet, the children actually stored more grams of nitrogen. These values were no more variable than they had been on the first diet, but the correlation with intake was low. This may have been caused by the high values for subject E. Since his data were not included in those for the medium protein diet and since he may have been storing more nitrogen on account of his previous illness, table 4 also gives the statistical data on the figures for the other three children. On this basis, the correlation between intake and retention was practically the same as that on the medium protein diet. Thus, after equilibrium, the three children on the second experiment stored nitrogen in relation to intake variations.

During the high protein diet all of the children gained at a faster rate than they had during the medium protein diet.

TABLE 5
Differences per kilogram of body weight between metabolism data after equilibrium on medium and high protein diets

SUBJECT	DIET			OUTPUT						ABSORPTION		RETENTION	
	Nitrogen	Increase	Dry weight	Urine		Feces				Nitrogen	Proportion of intake	Nitrogen	Proportion of intake
				Nitrogen	Proportion of intake	Nitrogen per gram dried food	Dried weight	Nitrogen per gram dried feces	Nitrogen				
	mg.	%	mg.	mg.	%	mg.	mg.	%	mg.	mg.	%	mg.	%
V	167	37.1	400	150	0.6	0.51	9	7	160	157	0.3	7	— 0.3
C	160	33.9	100	150	2.4	0.22	5	3	155	155	0.7	5	— 0.7
J	149	33.7	100	132	1.7	0.18	2	2	135	146	— 0.9	14	0.9

Table 6 shows that for some children it was several times as much per day. The 3-year-old girls gained faster than did the older children although they did not store a larger proportion of the nitrogen intake. It must be remembered, however, that the children received these diets for relatively short periods of time. Therefore, the gain during the high protein diet may have been the result of the medium protein diet which was probably better balanced than the diet they had had previous to the study. Nevertheless, these data indicate, as did those of Wang and her associates ('29), that the addition of protein to the diet may produce more rapid gains in weight than a lower protein diet.

These data show that the high protein diet increased the variability of the data during the first three periods and produced greater retentions then than at any other time. After

TABLE 6
Average gain in weight per day

DIET	B	D	E	V	C	J
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Medium protein	1.43	0.95	3.81	4.29	2.38
High protein	5.33	4.67	0.00	4.58	8.75	8.75

equilibrium was established it had no effect on the variability of the data or on the correlation with intake, it increased the total amount but not the proportion of total nitrogen excreted, it increased the coefficient of digestibility of the protein because it altered the proportion of nitrogen excreted in the urine and feces, it increased the grams of nitrogen but not the percentage of the intake stored in the body, and it caused a greater gain in weight in the children.

Average values. The average of the retention data per kilogram for all the children for each separate period might rule out individual fluctuations and show general tendencies in the results as compared to the average diet fluctuations. These averages, shown in figure 2, would be possible because the children in each experiment received exactly the same foods per kilogram. It will be seen that on the medium

protein diet the intake and retention variations followed the same general trends, that is, the average retention values tended to fall when the average intake values fell and vice versa. Following the increase in diet nitrogen, the retention values were higher during the first period than at any other time and then they fell slightly and tended to follow the same general course as the intake values. Subject B., in experiment

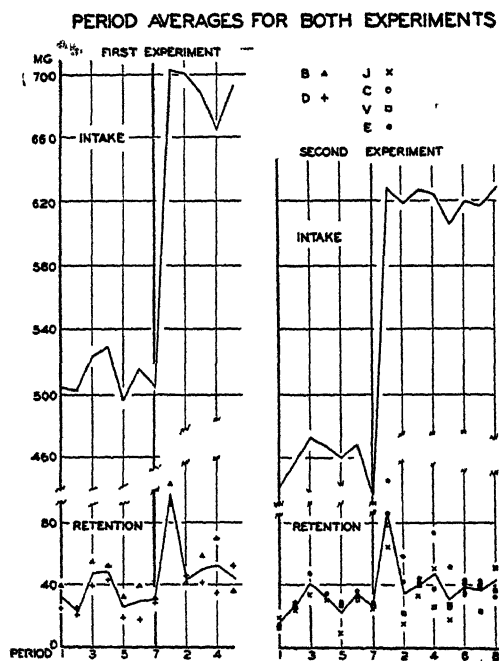


Figure 2

1, seemed to be an exception to this general statement since the average for the high protein diet in that experiment did not seem to follow the same trend. Nevertheless, the graft indicates that the children in the second experiment were in a fair degree of equilibrium during the last 15 and possibly during the last 21 days of the experiment, that they showed the same general trends and that they all tended to react in a similar manner under standard conditions.

SUMMARY

1. Five children received constant diets containing 3 gm. of protein per kilogram. They all excreted the same proportion of the nitrogen intake, but the urine figures fluctuated according to intake variations while the feces values remained constant for each child and were proportional to the dry weight of the feces. The ratio of fecal nitrogen to dry matter consumed remained fairly constant for each child but varied between children according to the character of the stools. For individual children the coefficient of digestibility varied between 86.0 and 92.7%, while the retention values remained more constant but both values fluctuated in proportion to the period by period variation in the diet.

2. Immediately following the change to a 4-gm. protein diet, the excretion values were irregular, but after 9 days they had apparently reached an equilibrium.

3. A comparison of the data following the preliminary period with that on the medium protein diet indicated the effects of protein on the metabolism. The percentage of the intake excreted did not vary, but the total grams increased, practically all of the increase occurring in the urine. The small fecal nitrogen increase was proportional to the increase in dry weight of the feces. The ratio of fecal nitrogen to dry food eaten increased slightly for each child but there was no correlation between the two factors. The coefficient of digestibility was higher for each child. The percentage of the intake nitrogen retained was practically the same, but the total grams increased.

4. The average of the retention and intake values per kilogram for all children for each separate period showed that the children retained nitrogen in relation to the period by period variations except immediately following the change in diet.

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HEAT PRODUCTION AND GASEOUS METABOLISM OF YOUNG MALE CHICKENS

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EIGHT FIGURES

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INTRODUCTION

Modern trends in poultry production have clearly emphasized the necessity of having precise and extensive data available on the energy metabolism of the domestic fowl. Such data are required by the agricultural engineer who is called upon to design the larger poultry plants wherein many thousands of broilers are to be raised, or an equally large number of pullets or hens are to be kept for egg production. However, the ultimate value of such data does not depend solely on their immediate practical utility, because they are also of considerable importance from the standpoint of comparative metabolism.

As has been pointed out by Benedict, Landauer and Fox ('32), who have made a comprehensive review of the literature on the energy metabolism of the domestic fowl, most of the investigations in this field up to the present time have been of a distinctly fragmentary nature. So far as the writers have been able to find, no extensive investigation is recorded in which simultaneous observations on the heat production and gaseous metabolism of the domestic fowl were made in an environment in which the temperature, the humidity, and

the concentration of oxygen and carbon dioxide were kept constant. In the present paper the writers present extensive data so obtained. They present the first curves ever published that show the course of the diurnal rhythm of oxygen consumption of the domestic fowl and how this diurnal rhythm changes with age. They also present some interesting data on the thermogenic effect of casein and gelatin in the domestic fowl.

APPARATUS AND METHODS

The Bureau of Animal Industry's respiration calorimeter at the National Agricultural Research Center, Beltsville, Maryland, was used in obtaining all the data reported in this paper. This respiration calorimeter is an instrument of precision; its construction, the accuracy attainable in its use, and the technic of operation have been discussed in detail by Barott ('37) and briefly by Barott, Byerly and Pringle ('36).

Male Rhode Island Red chickens hatched April 12, 1934, were used. They were separated from the females when 1 day old by the method of visual inspection of the copulatory organ described by Shrader, Burrows and Hammond ('34). After the segregation of the sexes, the males were placed in colony brooder houses on good range and fed a typical all-mash chick diet. The number of male chickens was sufficiently large to make it unnecessary to use the same ones in more than one experiment.

Thirty-five experiments were conducted in which simultaneous measurements of the energy and gaseous metabolism of a suitable number of chicks were made. The first experiment was begun when the chicks were 4 days old and the last when they were 130 days old. Each experiment was of 3 days' duration and when circumstances permitted two experiments were made each week.

The chickens to be used in each of the experiments were removed from the colony house at 8 A.M., brought to the laboratory, weighed, and placed in wire cages of such size as to fit the birds snugly and restrict movements to a minimum. The conditions to prevail during the experiment were then estab-

lished and the measurements of the gaseous metabolism were begun at 10 A.M. and of the heat production at 2 P.M.

At the beginning of the twenty-third hour the chickens were removed from the respiration chamber and weighed immediately. In most of the experiments the birds were then given water and fed a mixture of 98.5% of gelatin, 1.0% of starch and 0.5% of salt, or a similar mixture containing casein in place of the gelatin; but in six of the experiments the birds were given only water. The birds were then weighed a second time and placed again in the respiration chamber.

Measurements of the gaseous metabolism and heat production were begun again at 10 A.M. and 2 P.M., respectively. After the chickens had been under observation for a total period of 72 hours they were removed from the calorimeter, weighed, and returned to their colony houses.

When taken out of the respiration chamber at the beginning of the twenty-third hour, the younger chicks were allowed to eat as much as they would and the quantity of feed consumed was estimated by weighing the feed both before and after the chicks had eaten. After the birds were 6 weeks old a definite quantity of feed was given manually.

The mixture of gelatin, starch and salt contained 10.63% of moisture, 3.20% of ash, 0.11% of ether extractable material, and 15.20% of nitrogen; and the mixture of casein, starch and salt contained 7.46% of moisture, 2.70% of ash, 0.35% of ether extractable material, and 13.73% of nitrogen. Finely powdered salt and starch were added to the gelatin and casein because the resulting mixtures could be fed more easily than the pure gelatin or casein.

The conditions that prevailed in the calorimeter during each of the experiments were: temperature, 90°F.; relative humidity, 60%; oxygen content, 21%; and carbon dioxide content not exceeding 1%. The oxygen consumption of the chickens was determined for each 2-hour period that the chickens were in the calorimeter; carbon dioxide production was measured for the first period of 4 hours and then for each 8-hour period thereafter; direct measurements of heat production

were made during the 8-hour periods when carbon dioxide was being measured but not during the initial 4-hour period.

The respiration calorimeter was checked by alcohol and electric checks made at the beginning and end of the series of experiments. As a result of these checks, as well as of checks previously made, it was found that heat production, oxygen consumption, and carbon dioxide production could be measured with an error of less than $\pm 1\%$. All measuring apparatus was calibrated by the National Bureau of Standards.

PRESENTATION AND DISCUSSION OF THE DATA

The data obtained in this study of the heat production and gaseous metabolism of male Rhode Island Red chickens are too numerous to publish in detail, therefore only suitable summaries are presented.

BASAL METABOLISM

Mitchell and Haines ('27) reported that although the basal metabolism in chickens was often reached after a fasting period of 24 hours, a fast of 48 hours was required before the basal level was reached in all cases. Benedict, Landauer and Fox ('32) also reported that the average fowl must fast 48 hours before its metabolism may be considered as being at the basal level but they believed that if there is not a large quantity of feed in the crop at the beginning of the fast, 36 hours of fasting may suffice to reduce the metabolism to the basal level.

As will be shown later in this paper, the specifications of basal metabolism in the domestic fowl should include the time of day, as well as the number of hours elapsing after the last feed was eaten, because there is a rather pronounced diurnal rhythm of the energy metabolism in this species. The minimum metabolism during any 24-hour period is attained at approximately 8 P.M. and the maximum at approximately 8 A.M. The basal heat production of chickens is usually given on a 24-hour basis, although the measurements are made over a shorter period. If the mid-point of the observational period

is either 2 P.M. or 2 A.M., no serious error will result, but if the observational period is appreciably less than 24 hours and its mid-point is between 2 A.M. and 2 P.M., the estimated heat production for 24 hours will be greater than the true value. Likewise, if the mid-point of such a period is between 2 P.M. and 2 A.M., the estimated value will be less than the true value.

It seems best, therefore, to specify the basal heat production of the chicken as the average number of calories produced per hour during any period of adequate length, when the mid-point of this period is either 2 P.M. or 2 A.M. and occurs a suitable length of time after the last feed was eaten. The writers' data on the basal heat production and gaseous metabolism of male Rhode Island Red chickens are presented in figure 1. The plotted points represent the metabolism observed 66 hours after the last mixed feed had been eaten; but in some cases relatively small quantities of casein or gelatin were fed during the twenty-third hour. In all cases the mid-point of the observational period was 2 A.M. In this figure the metabolism is plotted against age but, inasmuch as the rate of growth is dependent to a considerable extent on the diet fed, the average live weights of the chickens after they had been fasted 66 hours are also plotted against age so that the metabolism for any given live weight, within the range studied, may be read from the plotted curves.

It is of interest to compare the data presented in figure 1 with those published by other investigators. Benedict, Landauer and Fox ('32) did not give the age of their Rhode Island Red chickens, they merely stated that they were hens and cocks; however, most of their birds were heavier than those used by the writers. Nevertheless, it is worth commenting that the oxygen consumption per gram of live weight of their lighter birds was only about two-thirds as great as that observed for the heaviest birds studied by the writers.

Brody ('30) has published some data on the heat production of Rhode Island Red chicks 3 to 56 days old. He computed the heat production from data obtained on oxygen consumption. Using his conversion factor the writers have computed

the oxygen consumption per gram of live weight. The resulting values showed no consistent change with age.

The writers also computed the oxygen consumption per gram of live weight of some White Plymouth Rock chickens studied by Mitchell, Card and Haines ('27). The chickens

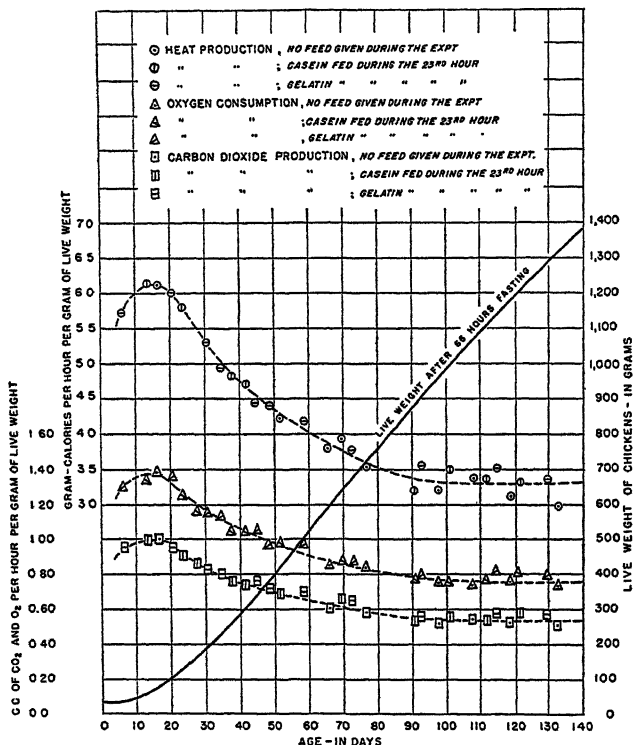


Fig.1 Effect of age on the average basal energy and gaseous metabolism of male chickens between 38 and 46 hours after feeding casein or gelatin, and/or between 62 and 70 hours after last mixed feed was consumed.

varied in age from 37 to 355 days. The computed values of the oxygen consumption agreed very closely with those observed by the writers, except in the case of the chickens which were 37 days old.

Mitchell, Card and Haines ('27) also obtained a few data on the oxygen consumption of Rhode Island Red chicks and

from these data computed the heat production. From the supplementary information they supplied on age and live weight, one must conclude that these chicks had grown rather slowly. The average weight of the chicks was only 239 gm. at an average age of 55 days, whereas, after 66 hours of fasting, the chicks used by the writers weighed 465 gm. at that age. However, they report that the average basal heat production of their 239-gm. chicks was 132 kg.-calories per 24 hours, per kilogram of live weight and, as indicated in figure 1, the writers observed an average value of 120 kg.-calories for fasted chicks of the same live weight. On an age basis the agreement is very poor but on a live weight basis it is much better.

An inspection of figure 1 shows that the basal metabolism per gram of live weight increased up to the age of about 15 days—or an average live weight of about 70 gm.—and then decreased to the age of about 100 days—or an average live weight of about 980 gm.—and that after this age was reached the metabolism was fairly constant, at least to the age of 133 days—or an average live weight of 1320 gm. The extent of the decrease in the basal heat production between the ages of 15 and 100 days is indicated by the fact that the basal heat production at the latter age was only about 54% as great as it was at the former age.

MAXIMUM RESTING METABOLISM

For the purpose of factoring into its component parts the total heat production of an animal, it is desirable to have information on the resting metabolism following the ingestion of feed. Accordingly, the data obtained by the writers were examined to determine 1) the relationship between the maximum resting metabolism at 8 A.M.,¹ following the ad libitum ingestion of feed, and the basal metabolism, and 2) the relationship between the average resting metabolism between 8 A.M. and 8 P.M. and the basal metabolism. These relationships are shown in table 1.

¹ The values at 8 A.M. are instantaneous values which were obtained by extrapolation.

It will be seen from the data presented in table 1 that in the very young chick the maximum resting metabolism, after the ad libitum ingestion of feed, is approximately 60% greater than the basal metabolism, whereas when the chick is between 16 and 20 weeks old it is only about 25% greater. However,

TABLE 1

The relationship between the resting energy metabolism, following the ad libitum ingestion of feed, and the basal energy metabolism

AGE	RESTING METABOLISM AT 8 A.M. ¹ AS PER CENT OF THE BASAL METABOLISM	AVERAGE METABOLISM BETWEEN 8 A.M. AND 8 P.M. AS PER CENT OF THE BASAL METABOLISM
<i>weeks</i>	%	%
0.5	160	130
1	150	123
2	138	120
3	136	119
4	135	118
5	135	118
6	134	118
7	133	117
8	133	117
9	132	117
10	131	116
11	130	115
12	129	115
13	128	114
14	126	112
15	125	111
16-20	125	109

¹ See footnote 1 on preceding page.

the average metabolism between 8 A.M. and 8 P.M. is only about 30% greater than the basal metabolism in the very young chick and only about 9% greater when the chick is between 16 and 20 weeks old.

R.Q.'S, CO₂ AND O₂ THERMAL QUOTIENTS, AND WATER ELIMINATION

In using the respiratory quotient as an indication of the type of material being metabolized by the chicken, it is necessary to keep in mind that the chief end-product of protein metabolism in this species is uric acid and not urea. Henry, Magee and Reid ('34) appear to have been the first to call

attention to this point. Benedict, Landauer and Fox ('32) and Mitchell and Haines ('27) seem to have ignored the effect on the respiratory quotient of this peculiarity of the protein metabolism in the chicken.

The writers have used the data given by Loewy ('23) on the metabolism of protein and the data of Coulson and Hughes ('30) on the composition of hen urine to estimate the heat production, oxygen consumption, and carbon dioxide and water production resulting from the metabolism of 1 gm. of protein

TABLE 2
Comparison of protein metabolism in the mammal and the bird

CLASS	HEAT PRODUCTION PER GRAM	O ₂ CONSUMPTION PER GRAM	CO ₂ PRODUCTION PER GRAM	H ₂ O PRODUCTION PER GRAM	RESPIRATORY QUOTIENT ($\frac{CO_2}{O_2}$)	CO ₂ THERMAL QUOTIENT ($\frac{KG.-CALS.}{GRAMS OF CO_2}$)	O ₂ THERMAL QUOTIENT ($\frac{KG.-CALC.}{GRAMS OF O_2}$)
Mammalia	<i>kg.-cals.</i> 4.3160	<i>cc.</i> 966.3	<i>cc.</i> 733.9	<i>gm.</i> 0.3960	0.801	2.838	3.124
Aves	3.84	891.1	628.6	0.47	0.705	3.11	3.02

TABLE 3
The heat production per gram and the respiratory and thermal quotients for the metabolism of protein, fat, and starch in the bird

MATERIAL METABOLIZED	HEAT PRODUCTION PER GRAM (KG.-CALC.)	RESPIRATORY QUOTIENT ($\frac{CO_2}{O_2}$)	CO ₂ THERMAL QUOTIENT ($\frac{KG.-CALC.}{GRAMS OF CO_2}$)	O ₂ THERMAL QUOTIENT ($\frac{KG.-CALC.}{GRAMS OF CO_2}$)
Protein	3.84	0.705	3.110	3.018
Fat	9.4610	0.707	3.373	3.279
Starch	4.1825	1.000	2.568	3.531

in the chicken. These estimates are given in table 2 along with Loewy's values for the mammal. For ready reference, the respiratory and thermal quotients for the metabolism of protein, fat and starch in the bird are given in table 3.

In figure 2 the basal CO₂ production observed between 10 P.M. and 6 A.M. of the third day in the several experiments is plotted against the O₂ consumption; and, for comparison, similar data obtained between 2 P.M. and 10 P.M. of the first day are also plotted. Although the ages of the chickens used

in these experiments ranged from 4 to 133 days and although the gaseous metabolism per gram of live weight varied over a rather wide range, the R.Q.'s showed relatively little variation within each of these two periods. The mean basal R.Q. was 0.719 ± 0.004^2 and the mean R.Q. during the period between 2 P.M. and 10 P.M. of the first day was 0.788 ± 0.005 .

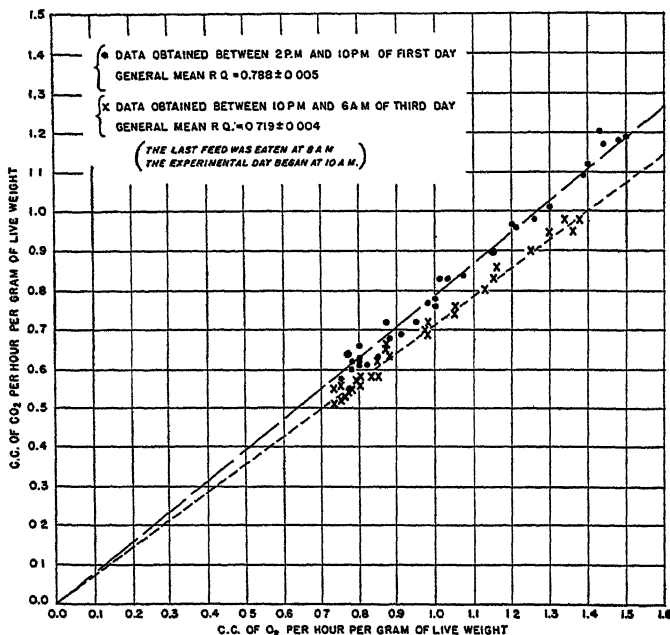


Fig. 2 CO₂ production plotted against O₂ consumption for all ages studied.

When the CO₂ production and the O₂ consumption, respectively, were plotted against the heat production, similar results were obtained (figs. 3 and 4). The mean thermal equivalents of CO₂ and O₂ were found to be 6.091 ± 0.038 and 4.377 ± 0.032 kg.-calories per liter, respectively, when the metabolism was at the basal level. During the period between 2 P.M. and 10 P.M. of the first day the mean thermal equivalents of CO₂ and O₂ were 5.909 ± 0.041 and 4.653 ± 0.031 kg.-calories per liter, respectively. The mean CO₂ thermal quo-

² Standard errors are used throughout this paper.

tients, when the metabolism was at the basal level and during the period between 2 P.M. and 10 P.M. of the first day, were 3.10 ± 0.02 and 3.01 ± 0.02 , respectively.

Brody ('30) adopted the value of 4.825 kg.-cals. per liter (R.Q. = 0.82) as the thermal equivalent of oxygen for normally fed animals about 12 hours after feeding. He also

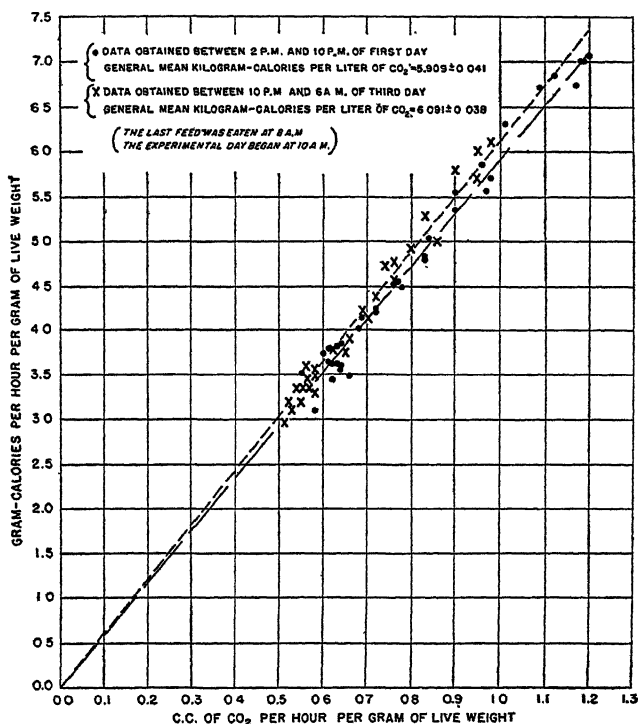


Fig. 3 Heat production plotted against CO_2 production for all ages studied.

concluded that this value may involve an error of 3%. For similar conditions, i.e., 10 hours after feeding, the writers obtained with chickens a mean value of 4.653 ± 0.031 kg.-cals. per liter (R.Q. = 0.788 ± 0.005). This value is only about 3.5% less than the value adopted by Brody.

According to the respiratory and thermal quotients, the chickens were metabolizing mostly protein³ when their metabolism was at the basal level. However, during the period between 2 P.M. and 10 P.M. of the first day, when the chickens were still in the absorptive stage, they were metabolizing an appreciable quantity of carbohydrate.

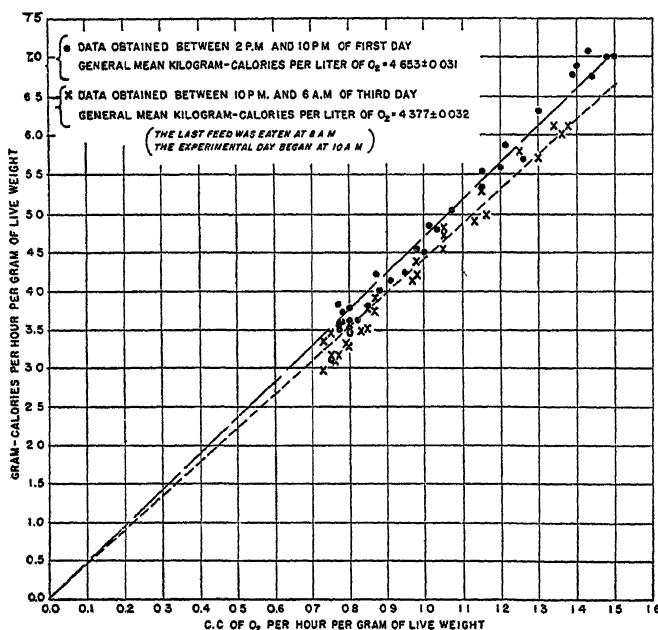


Fig. 4 Heat production plotted against O_2 consumption for all ages studied.

³ Inasmuch as the writers made direct measurements of both the heat production and gaseous metabolism, they were able to estimate from the R.Q. and the CO_2 and O_2 thermal quotients the relative quantities of carbohydrate, fat, and protein being metabolized. One method of estimation was as follows: If x, y and z represent, respectively, the percentages of carbohydrate, fat, and protein being metabolized, the following equations may be set up and readily solved:

$$\begin{aligned} 1.000 x + 0.707 y + 0.705 z &= 100 \times \text{the observed R.Q.} \\ 2.568 x + 3.373 y + 3.110 z &= 100 \times \text{the observed } CO_2 \text{ thermal quotient.} \\ 3.531 x + 3.279 y + 3.018 z &= 100 \times \text{the observed } O_2 \text{ thermal quotient.} \end{aligned}$$

The average numerical values of the R.Q., CO_2 thermal quotient, and O_2 thermal quotient of the chickens when their metabolism was at the basal level were 0.719, 3.100 and 3.063, respectively. If the equations given above are solved after these values are inserted in the proper places, it will be found that approximately 5% of carbohydrate, 10% of fat and 85% of protein were being metabolized.

Benedict, Landauer and Fox ('32) referred to the 'fat quotient' of the basal state and implied that birds in the basal state metabolize chiefly fat. Also, Mitchell and Haines ('27) concluded, on the basis of the respiratory quotients which they obtained, that "the metabolism of the chicken in the period from the forty-eighth to the seventieth hours after feeding is almost entirely at the expense of fat." That either Benedict, Landauer and Fox's or Mitchell and Haines' chickens, when in the basal state, metabolized very much fat is questionable. The writers observed almost exactly the same R.Q. for birds in the basal state as did Mitchell and Haines but the thermal quotients observed by the writers clearly indicate that only a small quantity of fat was being metabolized.⁴

The total water elimination, when expressed as milligrams per gram of live weight, was found to be fairly constant for all ages. It was 2.8 ± 0.1 mg. per hour per gram of live weight when the chickens were at the basal level and 3.1 ± 0.1 mg. per hour per gram during the 8-hour period between 2 P.M. and 10 P.M. of the first day of the experiments.

DIURNAL RHYTHM OF THE ENERGY METABOLISM

The course of the diurnal rhythm of the energy metabolism of the chicken, as it changes from hour to hour during a period of 24 hours, has not previously been determined.

Benedict, Landauer and Fox ('32) made day and night measurements of the gaseous metabolism of chickens and found that the values obtained during the day were approximately 10% greater than those obtained during the night. Brody ('30) made some early morning and late evening observations on the oxygen consumption of fasting chickens. His results were somewhat erratic since some of the evening values were greater than the morning values. However, in general, he obtained the larger values during the morning.

Mitchell, Card and Haines ('27) also observed higher values for the oxygen consumption during the day than they did at night. They at first thought that this difference in metabolism was a diurnal rhythm phenomenon but finally concluded

⁴ See footnote 3 on preceding page.

That there is a very definite rhythm in the oxygen consumption of chickens is clearly shown in figure 5, in which data from several of the writers' experiments have been plotted. The marked break in each of the first two curves at the end of the first 24 hours was caused by the protein that was fed during the twenty-third hour.

The effect of age on the diurnal rhythm is indicated in figure 5 but is shown more clearly in figure 6, in which the average amplitude of the rhythm for several consecutive experiments is plotted against the average age of the chicks. At the average age of 1 week the amplitude is about 12% of the oxygen consumption at 8 A.M. This means that an average maximum difference of about 24% was observed between the

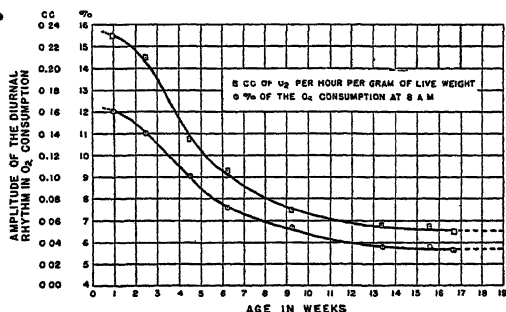


Fig. 6 Effect of age on the amplitude of the diurnal rhythm of the oxygen consumption per gram of live weight of male chickens.

oxygen consumption at 8 A.M. and at 8 P.M. After the age of 14 weeks was reached the amplitude of the diurnal rhythm tended to remain constant at a value of about 5.7% of the oxygen consumption at 8 A.M. This is equivalent to a difference of about 11.4% between the 8 A.M. and 8 P.M. values for oxygen consumption. These differences between the morning and evening results are of the same order of magnitude as those reported by Mitchell, Card and Haines ('27).

In view of the fact that the energy metabolism of the chicken exhibits a definite diurnal rhythm of considerable magnitude it is clear that specifications of the basal metabolism in the domestic fowl should include the time of day, as well as the number of hours elapsing after the last feed was eaten.

THE THERMOGENIC EFFECTS OF CASEIN AND GELATIN

The various theories of thermogenic action or, as it is more commonly called, specific dynamic action (SDA), have recently been reviewed by Borsook ('36), Wilhelmj ('35), Brody ('34) and Brody and Proctor ('33). In view of the fact that protein metabolism follows different courses in the mammal and the bird, comparative studies of SDA in these two classes should be of value in checking some of the theories.

Only a few studies have been reported on the thermogenic effect of feeding stuffs in the chicken and these have been reviewed by Mitchell and Haines ('27). In these studies, and in those of Mitchell and Haines ('27), corn was the only feeding stuff used. There appears to be no record of experiments in which the SDA of casein and gelatin was determined in the chicken; accordingly, the data obtained by the writers are of some interest.

Method of measuring SDA. When the total oxygen consumption, for each 2-hour period of the six experiments in which the chickens were fasted, was expressed as per cent of the total oxygen consumption at 8 A.M. of the first day and then plotted against the number of hours elapsing after the last feed was consumed, it was found that the resulting six curves very nearly coincided. Accordingly, the values for the total oxygen consumption for each of the corresponding periods during the last 48 hours of the fasting experiments were expressed as per cent of the 8 A.M. value, averaged, and then plotted against the number of hours elapsing after the last feed was consumed. Since no feed was fed during the first 22 hours in any of the experiments, the appropriate data from the last eighteen experiments were used to complete the curve for this period. The resulting composite curve is shown in figure 7. This curve, therefore, shows how the total oxygen consumption of fasting chickens, when expressed as per cent of the total oxygen consumption at the beginning of the fast, changed as the fast continued.

By using the data plotted in figure 7 it was possible to compute for those experiments, in which casein or gelatin was fed

during the twenty-third hour, what the oxygen consumption would have been if the casein or gelatin had not been fed. The difference between the total quantity of oxygen actually consumed and that which would have been consumed, if no casein or gelatin had been fed, was considered as a measure of the thermogenic effect of the protein fed.

The procedure followed in estimating the SDA of the casein and gelatin is illustrated in figure 8. The upper curve in each of the two pairs of curves represents the observed oxygen consumption and the lower curve represents the oxygen consumption which would have been observed if no protein had

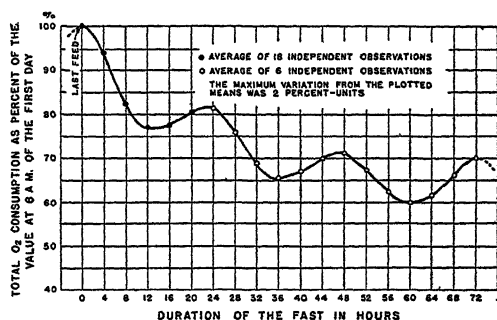


Fig. 7 Change in the total energy metabolism (as measured by the oxygen consumption), during a 3-day fast, of male chickens which were between 63 and 131 days old at the beginning of the fast.

been fed. For the first 24 hours, in the case of the lower curve of each pair, both the observed and computed oxygen consumption have been plotted to show the agreement between the two.

The rate of decrease of the oxygen consumption during the last 48 hours was always greater for the birds which were fed during the twenty-third hour than it was for the birds which were not fed. Accordingly, it was found that the curve representing the observed oxygen consumption and the curve representing the oxygen consumption which would have been observed, if no protein had been fed, eventually coincided. In the case of the younger birds the two curves coincided about

36 hours after feeding casein and about 48 hours after feeding gelatin. As the age of the birds increased the length of time required for the curves to coincide tended to increase, but it was always about two-thirds as great when casein was fed as when gelatin was fed.

After the observed and computed oxygen consumption had been plotted, as shown in figure 8, the area between the two

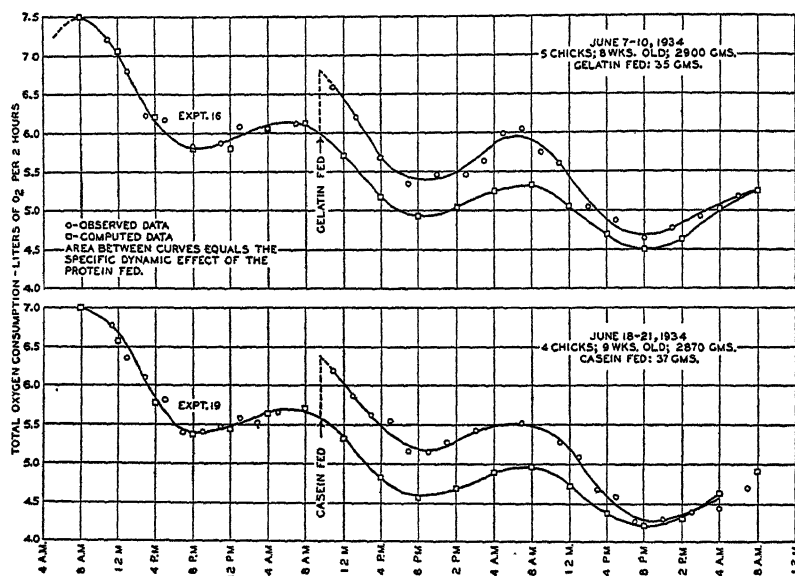


Fig. 8 Oxygen consumption during experiments 16 and 19, as observed for male chickens fed gelatin and casein, respectively, and as computed for fasting chickens of the same age.

resulting curves was carefully measured with a polar planimeter. The resulting estimates of the SDA of casein and gelatin in sixteen of the experiments are presented in table 4.

An examination of the data in table 4 shows that both the average initial increase in oxygen consumption and the average total increase per gram of nitrogen were greater for casein than for gelatin. If one uses 4.52 kg.-cal. per liter ⁵

⁵ An average of the thermal equivalents which were observed a) 10 hours after the last mixed feed was fed and b) when the metabolism was at the basal level.

TABLE 4

The specific dynamic action (SDA) of the two proteins, casein and gelatin, when fed to male chickens of different ages between 40 and 128 days, as measured by the oxygen consumption¹

EXPERIMENT NO.	AGE OF CHICKS	NUMBER OF CHICKS	TOTAL WEIGHT OF THE CHICKS WHEN THE PROTEIN WAS FED	QUANTITY OF NITROGEN IN THE PROTEIN FED			TOTAL O ₂ CONSUMPTION DURING 2-HOUR PERIOD BEFORE THE PROTEIN WAS FED	INCREASE IN O ₂ CONSUMPTION DURING FIRST 2-HOUR PERIOD AFTER PROTEIN WAS FED				SDA	
				Total	Per chick	Per kilogram of live weight		liters	liters	liters	%	Total increase in O ₂ consumption in O ₂ consumed before feeding	Total increase in O ₂ consumption per gram of nitrogen
Casein													
11	40	6	2.08	4.12	0.69	1.98	5.5	1.0	0.24	18	8.33	2.02	
14	50	6	3.04	5.49	0.92	1.81	6.8	1.2	0.22	18	12.54	2.28	
17	61	5	2.75	5.49	1.10	2.00	5.9	1.0	0.18	17	12.20	2.22	
19	68	4	2.78	5.08	1.27	1.83	5.7	0.8	0.16	14	10.28	2.02	
23	89	4	5.00	6.87	1.72	1.37	8.2	1.1	0.16	13	14.33	2.09	
26	99	4	4.32	8.92	2.23	2.06	7.8	1.4	0.16	18	19.24	2.16	
29	110	3	3.56	10.30	3.43	2.89	5.8	1.3	0.13	22	21.85	2.12	
32	120	2	3.24	8.24	4.12	2.54	5.2	1.1	0.13	21	18.64	2.26	
Mean	1.1	...	18	2.15	
Gelatin													
12	43	6	2.53	4.56	0.76	1.80	6.1	0.8	0.18	13	8.62	1.89	
13	47	6	2.73	4.56	0.76	1.67	6.3	0.8	0.18	13	8.70	1.91	
16	57	5	2.90	5.32	1.06	1.83	6.1	0.8	0.15	13	10.33	1.94	
20	71	4	3.26	6.08	1.32	1.87	6.5	0.9	0.15	14	12.18	2.00	
24	92	4	4.00	9.12	2.28	2.28	6.8	0.9	0.10	13	18.46	2.02	
27	103	3	3.17	9.12	3.04	2.88	5.5	0.9	0.10	16	17.21	1.89	
30	113	2	2.52	9.88	4.94	3.92	4.1	0.8	0.08	20	18.15	1.84	
34	128	2	2.60	9.12	4.56	3.51	3.9	0.8	0.09	21	17.28	1.89	
Mean	0.8	...	15	1.92	

¹ In the first ten experiments, the ad libitum consumption of casein and gelatin was small and irregular and so the data obtained on SDA in these experiments are not given. In the subsequent experiments with casein and gelatin the chickens were forcibly fed. In experiments 18, 21, 25, 28, 31 and 35 the chickens were fasted for 3 days and, hence, no casein or gelatin was fed. It was not possible to complete experiments 15, 22 and 33.

as the average thermal equivalent of oxygen after casein or gelatin was fed, it is found that the average SDA of casein was 9.7 kg.-cal. per gram of nitrogen and of gelatin 8.7 kg.-cal. per gram of nitrogen. Apparently the SDA of casein and gelatin was not affected by age, although the duration of the SDA was longer in the older chickens than it was in the younger ones.

Attention is directed to the fact that the SDA, its initial intensity, and its duration are not the same for casein and gelatin. The duration of the SDA was not so long for casein as it was for gelatin, but the initial oxygen consumption during the first 2 hours reached higher levels after casein was ingested than it did after gelatin was ingested. The difference in SDA between casein and gelatin is fairly large and, statistically, it is highly significant.

Although no determinations of the nitrogen excretion of the chickens were made, it is apparent that, in effect, the method of computing SDA used by the writers was essentially the same as that recommended by Borsook ('36), inasmuch as in both methods the SDA may be expressed as the excess calories of heat produced per gram of nitrogen in the protein fed. Accordingly, it must be concluded that, in general, the SDA of casein and gelatin in the chicken is not so great as it is in man or the dog, because, according to Borsook's ('36) calculations, the SDA of casein in man was between 14 and 21 kg.-cal. per gram in Gigon's experiments and the SDA of gelatin in the dog was between 12 and 15 kg.-cal. per gram in Rapport and Beard's experiments.

SUMMARY AND CONCLUSIONS

Thirty-five experiments, each of 72 hours duration, were made in which energy and gaseous metabolism of male Rhode Island Red chickens between the ages of 4 and 133 days was measured. The conditions prevailing in the calorimeter during each experiment were: temperature, 90°F.; relative humidity, 60%; O₂ content, 21%; and CO₂ content, not exceeding 1%. The oxygen consumed during periods of 2 hours and the

carbon dioxide and heat produced during periods of 8 hours were determined. In some of the experiments either casein or gelatin was fed during the twenty-third hour in order to study the thermogenic effect of these two proteins.

Data on basal metabolism, maximum resting metabolism, R.Q.'s, CO_2 and O_2 thermal quotients and equivalents, total water elimination, diurnal rhythm, and the thermogenic effects of casein and gelatin are given. Curves are presented that show the course of the diurnal rhythm of oxygen consumption in the domestic fowl, and how the amplitude of this diurnal rhythm changes with age.

It is concluded that:

1. The basal energy metabolism per gram of live weight, after 66 hours of fasting, is greatest in the male Rhode Island Red chicken when it is about 15 days old or when the weight is about 70 gm. Thereafter the basal energy metabolism decreases at a rapid rate until it becomes relatively constant at an age of about 100 days, or an average weight of about 980 gm.

2. In the very young chick the maximum resting metabolism after the ingestion of feed is about 60% greater than the basal metabolism, whereas between the ages of 16 and 20 weeks it is only about 25% greater.

3. The basal R.Q. is very nearly the same for all ages between 4 and 133 days; it is 0.719 ± 0.004 .

4. The total water elimination per gram of live weight was fairly constant at all ages studied. It was 2.8 ± 0.1 mg. per hour per gram when the chickens were at the basal level and 3.1 ± 0.1 mg. per hour per gram during the 8-hour period between 2 P.M. and 10 P.M. of the first day of the experiment.

5. The thermal equivalent of oxygen in the chicken is 4.653 ± 0.031 kg.-calories per liter about 10 hours after the last ingestion of feed; and 4.377 ± 0.039 kg.-calories per liter when the fasting metabolism is at the basal level.

6. The maximum energy metabolism occurs at about 8 A.M. and the minimum at about 8 P.M.

7. The amplitude of the diurnal rhythm of the energy metabolism is greatest in the very young chick and decreases rapidly with age.

8. In the chicken the SDA of casein is about 9.7 kg.-calories per gram of nitrogen and that of gelatin is about 8.7 kg.-calories per gram of nitrogen. The difference is statistically significant.

9. The SDA of casein and gelatin in the growing chicken is apparently not affected by age but the duration of the SDA is longer in the older chickens than it is in the younger ones, and longer for gelatin than it is for casein.

10. The SDA of casein and gelatin in the chicken is not so great as it is in man or the dog.

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THE TRACE ELEMENT CONTENT OF THE NEWBORN RAT (AS DETERMINED SPECTROGRAPHICALLY)

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So far as could be determined by a search of the literature, observations of trace elements in the normal newborn rat have been reported for only a few elements, namely; aluminum (Myers and Mull, '28), cobalt (Stare and Elvehjem, '33), copper (Lindow, Peterson and Steenbock, '29), manganese (Skinner, Peterson and Steenbock, '31; Orent and McCollum, '31), and zinc (Thompson, Marsh and Drinker, '27; Bertrand and Beauzumont, '30; Newell and McCollum, '33).

Lack of suitable methods prior to the extensive use of spectroscopy made difficult the determination of more than one trace element at a time in the ash obtained from an individual newborn rat.

This paper reports the spectrographic estimation of trace elements in the ash of the newborn rat.

EXPERIMENTAL

The rats (*Mus norvegicus*) used in this study were propagated from a foundation colony secured from Dr. L. S. Palmer, University of Minnesota in 1936. The stock diet consisted of a commercial fox feed ¹ which was fed 'ad libitum,' supplemented daily by 20 cc. of fresh whole milk, and lettuce twice weekly. Ordinary distilled water was available to the animals at all times.

¹Champion 100% fox feed. Ingredients, dehydrated meat, skim milk powder, oat meal, yellow corn meal, bran, red dog flour, second clear flour, pure wheat germ, steamed bonemeal, calcium carbonate, iodized salt and cod liver oil. Analysis, moisture 6.7%, protein 31.0%, fat 8.0%, ash 7.0%, fiber 4.8%, and nitrogen free extract 42.5%.

Prior to parturition the mothers were placed in individual cages provided with wood shavings. The young were taken as soon as possible after birth, association with the mother never exceeding 1 day.

The newborn were brushed free from any wood shavings, rinsed with redistilled water, and placed in platinum dishes for drying at 100°C. All glassware and platinum used in the preparation of the ash was leached with hot 1:1 hydrochloric acid and rinsed several times with water, triple-distilled through a silica still. After drying, the animals were ashed in a muffle furnace at a temperature not exceeding 450°C. for 24 to 48 hours. The ash was then homogenized in an agate mortar and analyzed spectrographically.

Spectrographic technic

The procedure was designed to permit an estimation of the proportions of certain elements present in magnitudes detectable by spectrography on the original sample.

A Littrow spectrograph with a linear dispersion of about 30 inches between 2250 Å and 5500Å was used. Two prisms were used with this instrument, a glass prism for lines of wave length greater than 3800 Å and a quartz prism for shorter wave lengths.

A small aliquot of the homogenized ash was volatilized in a 220 volt arc using a current of 9 to 10 amperes. Specially purified graphite was used as electrodes. Repeated spectra of the graphite electrodes were made to insure a control of electrode impurities. In taking the spectrum of the sample, the arc was maintained until the sample was completely volatilized. Incomplete volatilization would permit fractionation, involving a possible retention of the higher boiling elements in the residue. This might vitiate estimates of the amounts of the elements present.

A mixture of the twenty-four elements included in the analysis was used as a wave length standard, by means of which the lines in the spectra of the sample were identified.

The estimations were made by the comparison method used by Nitchie ('29). Standard powders containing known per-

centages of the various elements were spectrographed in juxtaposition with spectrograms of the ash samples. The percentages of the elements present were then estimated by comparing visually the intensities of spectral lines in the sample with corresponding lines in the standards. Duplicate determinations were made on each sample.

The data thus obtained are not intended as precision determinations, but are indicative of the 'order of magnitude' of the proportions of the elements present. To avoid misunderstanding as to precision, and also to retain a legitimate basis for comparison, the data are presented in range form. (For example, 0.001–0.005 recorded in the table should be read: The amount of the element in the sample lies between 0.001 and 0.005 mg.)

The approximate sensitivity of the method is: between 0.0001 and 0.001% for chromium, cobalt, copper, nickel and silver; between 0.001 and 0.01% for aluminum, barium, beryllium, manganese, molybdenum, lead, strontium, tin, titanium, vanadium and zinc; between 0.01 and 0.1% for antimony, bismuth, boron, cadmium, lanthanum, thallium, yttrium and zirconium.

In view of the varying sensitivities listed above, the term 'trace' used in the table has a varying significance. In all cases, the term 'trace' corresponds to the lower limit of sensitivity for the element involved. Thus a trace of chromium would signify about 0.001%, whereas a trace of boron would signify about 0.01%.

RESULTS

The results of the spectrographic analyses of fourteen newborn rats from six different litters (not more than three of which were from any one litter) and of the stock diet of the mothers are given in tables 1 and 2. The amount of the element present is expressed in milligrams per rat and in p.p.m. of the feed. The live weights of the animals, the ash weights, the percentage of moisture and the percentage of ash on the fresh basis also are recorded.

TABLE 1

Spectrographic estimation of aluminum, barium, chromium, copper, lead and manganese in newborn rats and in the stock feed of the mothers. The values are expressed in milligrams per rat and in p.p.m. for the stock feed on the fresh basis¹

RAT NO.	WEIGHT ² gm.	ASH gm.	MOISTURE %	ASH (WZP ³ BASIS) %	ALUMINUM	BARIUM	CHROMIUM	COPPER	LEAD	MANGANESE
2227	5.727	0.096	85.2	1.67	0.0008-0.003	0.001	0.001	0.008-0.03	0.0008-0.003	0.0008-0.003
2276	5.837	0.104	85.2	1.78	0.001	0.001	0.008-0.03	0.01-0.05	Tr	0.0003-0.008
2277	5.230	0.094	83.9	1.79	0.0009	0.0009	N.D.	0.007-0.03	0.0003-0.005	0.0009-0.005
2278	5.400	0.092	84.1	1.71	0.0009	0.0009	N.D.	0.007-0.03	0.0009	0.0007-0.003
2279	5.340	0.096	85.3	1.79	0.001	0.001	N.D.	0.03-0.08	0.001	0.001
2280	5.369	0.091	85.1	1.69	0.0007-0.003	0.0009	Tr	0.007-0.03	Tr	0.0007-0.003
2281	4.858	0.094	85.0	1.94	0.0008-0.003	0.0009	N.D.	0.008-0.03	0.0009	0.0009
2282	5.741	0.096	83.2	1.68	Tr	0.001	Tr	0.008-0.03	0.001	0.0008-0.003
2283	5.757	0.098	83.5	1.70	0.0008-0.003	0.001	0.001	0.05-0.1	0.0008-0.003	0.001
2284	5.020	0.094	85.4	1.87	0.0009	Tr	N.D.	0.009-0.05	0.0009	0.0009
2285	6.126	0.103	82.6	1.67	0.001	0.001	N.D.	0.03-0.08	0.001	0.001
2286	5.282	0.093	85.9	1.76	0.0007-0.003	0.0009	N.D.	0.007-0.03	?	0.0009
2287	5.522	0.095	85.5	1.72	0.001	0.001	N.D.	0.009-0.05	?	0.0008-0.003
2288	5.696	0.099	84.9	1.73	0.0008-0.003	0.001	Tr	0.008-0.03	N.D.	0.001
Stock feed	56.945	3.84	7.85	6.74	34.0-67.0	7.0-34.0	Tr	5.0-20.0	0.5-2.0	20.0-54.0

¹ The approximate sensitivity of the method is: for chromium, cobalt, copper, nickel and silver, between 0.0001 and 0.001%; for aluminum, barium, beryllium, manganese, molybdenum, lead, strontium, tin, titanium, vanadium and zinc, between 0.001 and 0.01%; for antimony, bismuth, boron, cadmium, lanthanum, thallium, yttrium and zirconium, between 0.01 and 0.1%. 'N.D.' denotes 'not detected.' 'Tr' denotes 'trace.' '?' denotes 'trace, but not positive identity.

TABLE 2

Spectrographic estimation of molybdenum, nickel, silver, strontium, tin and zinc in newborn rats and in the stock feed of the mothers. The values are expressed in milligrams per rat and in p.p.m. for the stock feed on the fresh basis¹

RAT NO.	WEIGHT ²	ASH	MOISTURE	ASH (WET BASIS)	MOLYBDENUM	NICKEL	SILVER	STRONTIUM	TIN ³	ZINC
	gm.	gm.	%	%						
2227	5.727	0.096	85.2	1.67	Tr	0.001	0.001	0.001	0.001	0.05-0.1
2276	5.837	0.104	85.2	1.78	N.D.	0.008-0.03	Tr	0.001	Tr	0.08-0.3
2277	5.230	0.094	83.9	1.79	N.D.	N.D.	Tr	0.0009	0.0009	0.05-0.09
2278	5.400	0.092	84.1	1.71	N.D.	N.D.	Tr	0.0009	0.0008-0.003	0.07-0.3
2279	5.340	0.096	85.3	1.79	N.D.	N.D.	N.D.	0.001	0.001	0.08-0.3
2280	5.369	0.091	85.1	1.69	N.D.	N.D.	Tr	0.0009	0.0008-0.003	0.07-0.3
2281	4.858	0.094	85.0	1.94	N.D.	N.D.	Tr	0.0009	0.0009	0.07-0.1
2282	5.741	0.096	83.2	1.68	N.D.	N.D.	Tr	0.001	0.001	0.05-0.09
2283	5.757	0.098	83.5	1.70	N.D.	0.001-0.005	Tr	0.001	0.0008-0.003	0.08-0.3
2284	5.020	0.094	85.4	1.87	N.D.	N.D.	N.D.	Tr	0.0009	0.08-0.3
2285	6.126	0.103	82.6	1.67	N.D.	N.D.	Tr	0.001	0.0008-0.003	0.08-0.3
2286	5.282	0.093	85.9	1.76	N.D.	N.D.	Tr	0.0009	0.0009	0.08-0.3
2287	5.522	0.095	85.5	1.72	N.D.	N.D.	N.D.	0.001	Tr	0.08-0.3
2288	5.696	0.099	84.9	1.73	N.D.	N.D.	N.D.	0.001	Tr	0.08-0.3
Stock feed	56.945	3.84	7.85	6.74	0.5-2.0	2.0-5.0	N.D.	0.7	Tr	67.0

¹ The approximate sensitivity of the method is: for chromium, cobalt, copper, nickel and silver, between 0.0001 and 0.001%; for aluminum, barium, beryllium, manganese, molybdenum, lead, strontium, tin, titanium, vanadium and zinc, between 0.001 and 0.01%; for antimony, bismuth, boron, cadmium, lanthanum, thallium, yttrium and zirconium, between 0.01 and 0.1%. 'N.D.' denotes 'not detected.' 'Tr' denotes 'trace.'

Aluminum, barium, copper, manganese, strontium, tin and zinc were present in all of the rat ashes. Lead was detected in eleven of the fourteen rat ashes; silver in ten; chromium in six; nickel in three; and molybdenum in one. The following elements were detected in the stock feed; aluminum, barium, chromium, copper, lead, manganese, molybdenum, nickel, strontium, tin, titanium and zinc. Antimony, beryllium, bismuth, boron, cadmium, cobalt, lanthanum, thallium, vanadium, yttrium, and zirconium were not detected in any of the ash samples. Titanium was not detected in the ash of the rats but a trace appeared in the ash of the feed.

DISCUSSION

Certain elements were detected in all of the ash samples, hence, the results for these elements are at least consistent. Other elements were detected irregularly in the animal ashes which might indicate that this lack of consistency is due either to contamination of the ash in some manner, or to differences in placental transmission.

In general, as pointed out previously, the small quantity of ash obtained from a normal newborn rat does not permit an extensive chemical analyses of the ash. Consequently, the data in the literature consist primarily of the determination of only one element in a given sample of ash. In the case of those elements which have been determined previously, the results, obtained in this study are in reasonable agreement with the published findings of other investigators.

Zinc. The value of the zinc content was the highest attained for any of the trace elements present in the ash of the normal newborn rat. Thompson, Marsh and Drinker ('27) reported a zinc content of 0.038 mg. per gram of rat; and Newell and McCollum ('33), using a spectrographic method, found 0.37 mg. per animal. Bertrand and Beauzemont ('30) reported 37.51 mg. of zinc per 100 gm. of dry total body tissue (1-day-old rats), which appear to be somewhat higher than the above values and those reported in this paper.

Copper. Copper was found in the next highest amount. Lindow, Steenbock and Elvehjem ('29) reported 0.0089 to 0.0134 mg. of copper per animal in their normal newborn rats.

Manganese. By means of spectrographic technic, Orent and McCollum ('31) found 0.1 to 0.5 p.p.m. of manganese in normal newborn rats, while Skinner, Peterson and Steenbock ('31) reported 0.0013 to 0.0019 mg. of manganese per rat.

Cobalt. Cobalt could not be detected by Stare and Elvehjem ('33) in newborn rats whose mothers were on an experimental diet free from cobalt. Neither was cobalt detected in the ashes analyzed in this study.

Aluminum. The values for aluminum found in this investigation, however, are much lower than those reported by Myers and Mull ('28), who found 0.33 mg. per 100 gm. of fetal young, and 0.20 mg. per 100 gm. of young (?) animal. The values are approximately 0.01 to 0.02 mg. per rat when computed on the basis of an approximate weight of 5 gm. for a newborn rat.

The following elements: barium, chromium, lead, molybdenum, nickel, silver, strontium, and tin were found in smaller quantities (traces). A search of the literature failed to disclose any of these as having been reported previously in the normal newborn rat.

The fact that twelve of the twenty-four elements studied could not be detected in the animal ashes does not mean that these were not present. They might be present in amounts smaller than the sensitivity of the method in use.

The common presence of aluminum, barium, copper, lead, manganese, strontium, tin and zinc in these ashes suggests that these elements are probably transmitted through the placenta.

Other elements detected irregularly in the ash samples, might also be involved in placental transmission; the evidence, however, is insufficient to warrant a definite conclusion.

SUMMARY

Spectrographic estimation of trace elements in normal newborn rats showed aluminum, barium, copper, manganese, strontium, tin and zinc, to be present in all of the animals. Lead and silver were detected in more than half the rat ash samples, Antimony, beryllium, bismuth, boron, cadmium, cobalt, lanthanum, thallium, titanium, vanadium, yttrium and zirconium were not detected in any of the rat samples.

It is suggested that the following elements might be transmitted from mother to young—aluminum, barium, copper, lead, manganese, strontium, tin and zinc.

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THE CHANGES IN TOTAL CALCIUM CONTENT OF THE BONES DURING THE DEVELOP- MENT OF RICKETS

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The metabolic disturbance leading to the development of rachitic bone changes is characterized by an impaired absorption of calcium and phosphorus from the intestinal tract. Whether the decreased absorption of calcium or that of phosphorus is of primary importance, and to what extent the one disturbance is dependent on or caused by the other, is still a moot question. The great difficulty of solving this problem by direct measurements of intake and output is vividly demonstrated in the recent papers of Nicolaysen ('37 a, b, c) and Innes and Nicolaysen ('37) where the question of absorption of exogenous calcium and phosphorus, of re-excretion into the intestines and further re-absorption is thoroughly discussed and further elucidated by ingenious experiments. This author concludes that, "in the rat . . . the action of vitamin D in the gut is confined to a direct action on the absorption of calcium. The well-known reduced absorption of phosphorus in vitamin D deficiency is due to a precipitation by the increased amount of calcium in the bowel." This statement would reinstate calcium into the role of prime offender, a role that it has not played since the discovery of the low serum phosphorus in rickets and the low phosphorus diet for the production of experimental rickets.

Even though the direct evidence may, perhaps, still be open to discussion, there is indirect evidence indicating that the

metabolic disturbance in rickets may be accompanied by a calcium deficiency. The enlargement (Erdheim, '14; Ritter, '20; Pappenheimer and Minor, '21) and increased function (Hamilton and Schwartz, '33) of the parathyroid glands is one such piece of indirect evidence; the association of rickets and tetany is another. If, however, an insufficient absorption of calcium from the intestine is a regular feature of the metabolic disturbance of rickets, then it becomes rather surprising that this deficiency does not always lead to a decrease in the calcium concentration of the serum, just as the decreased phosphorus absorption always seems to result in a fall in the concentration of serum phosphorus. We must, then, assume that the concentration of calcium is maintained at a normal level by some special mechanism, perhaps, for instance, by the parathyroid hyperactivity just referred to. No amount of parathyroid hyperactivity could, however, supply the tissue fluids with sufficient calcium, if the absorption from the gut were considerably decreased. All that the parathyroids could do would be to govern, in some way, the mobilization and distribution of some endogenous store of calcium.

The only store of calcium in the body is contained in the bones. The mobilization of bone calcium can be studied only by determining the changes in the total amounts of calcium in the bones. A change in the total amounts of minerals in a bone will take place when there is a difference between the amounts deposited in new bone and in the amounts liberated by the resorption of old bone. This difference, the mineral balance of the bone, has not the same significance as decalcification or increased density of the bones. A negative balance must always lead to decalcification, but so may a positive balance, if it is too small in proportion to the rate of growth of the bones.

It would, *a priori*, seem highly probable that the calcium balance of the bones in rickets is a negative one. The histological examination shows, in severe cases, no evidence of calcification, while bone resorption seems to continue at a normal or even increased rate (Eliot and Park, '37). This histological picture would indicate that the total amounts of calcium

in the skeleton are decreasing, and the purpose of the present work was to check up on the impression thus obtained; to determine the changes in total calcium content of the bones during the development of rickets.

METHODS

To solve this problem, the only completely satisfactory procedure would be to use some method by which the total calcium could be accurately determined in the skeleton of a living animal, then produce rickets in that animal and, after a suitable period, repeat the determination. As no such method

TABLE 1
Comparison of metatarsals of both legs removed at the same time

NO.	DIET	TOTAL Ca MILLIGRAMS			LENGTH, MILLIMETERS		
		Left	Right	Difference	Left	Right	Difference
51	Normal	52.0	51.2	0.8	19.0	18.9	0.1
54	Normal	80.8	80.8	0.0	23.2	23.1	0.1
61	Normal	60.0	61.8	1.8	21.0	21.1	0.1
62	Normal	96.8	95.2	1.6	20.8	20.8	0.0
65	Normal	43.2	42.5	0.7			
67	Normal	47.2	44.4	2.8	18.1	18.0	0.1
68	Rachitogenic for 1 week	49.2	50.4	1.2	20.1	20.1	0.0
72	Rachitogenic for 1 week	110.0	110.0	0.0	24.4	24.2	0.2
73	Rachitogenic for 1 week	41.6	41.0	0.6	19.8	19.9	0.1
75	Rachitogenic for 1 week	47.8	46.8	1.0	19.4	19.6	0.2

exists, we have instead, compared the calcium content of corresponding bones. The amputation of one leg is easily performed and is generally well tolerated by young rabbits; after a period on rachitogenic diet the animals may be killed and the bones of the other leg analyzed. The diet used for the production of rickets was the McCollum diet 3143 (McCollum et al., '21) and the degree of rickets was estimated by x-rays taken just before the animals were killed. The bones analyzed were the metatarsals, and it may be seen in table 1 that the right and left metatarsals, when removed at the same time, contain about equal amounts of calcium, the greatest difference being somewhat less than 3 mg. The difference

obtained when measuring the length of the bones was never greater than 0.6 mm.¹ In order to determine the effect of the amputation on growth and calcification, normal animals on stock diet and of approximately the same age as the experimental animals were operated upon and killed at the same time intervals as the latter.

The bones, after being dissected free from adhering soft tissue were dried at 110°C. and then ashed at 400°C. Calcium was determined by the method of Fiske and Logan ('31), phosphorus by the method of Fiske and Subbarow ('25).

TABLE 2
Change in metatarsals in first 4 weeks of rachitogenic diet

NO.	DEGREE OF RICKETS	TOTAL Ca MILLIGRAMS			LENGTH, MILLIMETERS		
		Initial	Final	Difference	Initial	Final	Difference
445	+++	102.7	108.0	+ 5.3	23.9	27.8	3.9
408	+++	99.9	102.3	+ 2.4	24.5	26.9	2.4
155	+	60.4	62.6	+ 2.2	20.7	22.2	1.5
153	+	92.0	86.8	- 5.2	22.4	23.6	1.2
164	±	114.2	101.5	-12.7	24.7	25.1	0.4
434	++	159.2	143.6	-15.6	26.8	27.6	0.8
159	+++	146.1	121.8	-24.3	25.1	25.8	0.7
417	+++	181.6	148.6	-33.0	27.8	29.4	1.6
7	Control	64.0	114.4	+50.4	17.0	23.5	6.5
173	Control	126.2	153.4	+27.2	22.7	28.3	5.6
166	Control	92.9	115.6	+22.7	22.7	25.1	2.4
10	Control	76.8	97.3	+20.5	18.6	25.0	6.4
2	Control	65.6	79.6	+14.0	19.2	26.0	6.8

RESULTS

In the first group of animals one leg was amputated on the day that the rachitogenic diet was started, then, after 4 weeks, the animals were killed and the second leg removed. The controls were animals of approximately the same age, who were operated on and then killed at the same time intervals as the experimental animals, but fed on standard stock diet. Table 2 shows that in these normal animals the total amounts

¹ This difference was observed in a case in which the analysis was lost and that, therefore, is not included in the table.

of calcium in the metatarsals increased appreciably during this period of 4 weeks and that the bones also showed a considerable increase in length. Of the rachitic animals, three showed a very slight increase of total calcium (in two of these the increase falls within the limit of error of the method as shown in table 1). In the remaining five animals the total calcium decreased, in some quite markedly. There is a certain rough relationship between the behavior of the total calcium and the increase in length of the bones, the best increase in length being found where there was no loss of calcium.

TABLE 3
Change in metatarsals from fourth to seventh week of rachitogenic diet

NO.	DEGREE OF RICKETS	TOTAL Ca MILLIGRAMS			LENGTH, MILLIMETERS		
		Initial	Final	Difference	Initial	Final	Difference
422	++++	135.2	133.6	- 1.6	30.0	32.2	2.2
145	+++ (healing)	165.6	145.6	-20.0	32.0	34.1	2.1
149	+++	125.0	101.0	-24.0	24.8	25.7	0.9
421	++ (healing)	126.6	83.6	-43.0	25.0	26.2	1.2
90	Control	166.0	211.0	+45.0	32.2	34.1	1.9
139	Control	147.8	180.4	+32.6	26.0	28.3	2.3
137	Control	217.0	235.0	+18.0	32.0	34.2	2.2

In the second group (table 3) the experimental animals were given rachitogenic diet for 4 weeks before the amputation was performed. The diet was continued for 3 weeks after the operation, then the animals were killed and the second leg removed. The animals in this group did not stand the operation as well as those in the previous group, and those reported in the table were the few survivors of a much greater number of rabbits operated upon. The controls were animals of approximately the same age, fed on a normal stock diet, operated and killed at the same time intervals as the experimental animals. The controls showed an increase of the total calcium of about the same magnitude as found in the previous group. Of the rachitic animals, two showed in the x-ray definite signs

of healing. This somewhat complicates the interpretation of the results, as the experiment, probably, has included a period both of progression and regression of the rachitic process. The degree of this process, as marked in the table by plus signs, indicates the degree of rickets which seemed to have been present before the healing began. Both these animals showed a marked loss of calcium. In the two remaining, both with marked rickets, we find one with practically no loss of calcium and a growth in length as good as that of the controls, while the other has lost a considerable amount of calcium and grown very little.

In all cases we have also determined the amounts of total phosphorus, but as all our determinations were made on the whole bone, including the marrow, we have considered the figures obtained only as a rough check on the determinations of calcium, and have not included them in the tables. We will mention, however, that as soon as the change in total calcium was more than 3 mg., the total phosphorus always showed a change in the same direction, with the exception of case 164, where the phosphorus showed an increase of 3.6 mg., while the calcium decreased.

COMMENT

The rachitic animals and the controls behaved differently as far as the total calcium content of the metatarsal bones is concerned. All the controls showed a considerable increase, while the rachitic animals either lost calcium or showed a very small, practically negligible, increase. This difference cannot, of course, be due to the amputation, as all the animals were subjected to this operation. We do not think that it was due to any difference in activity, as none was noted, and the amount of activity of any rabbit in the ordinary laboratory cage is always very small. The diet must be responsible for the loss of total bone calcium and as we have previously shown (Hamilton, Kajdi and Meeker, '30) that on this diet the rabbit does not develop any acidosis, which might have led to decalcification, we feel that the loss of calcium is, in some way, directly related to the fact that the animals developed rickets.

We assume that this negative mineral balance of the bones is due to a lack of calcification, while bone resorption continued at a more or less normal rate.

As rachitic animals continue to grow, there must be an increase in the total amounts of phosphorus contained in the soft tissues. The characteristic features of the phosphorus metabolism of the body in rickets would be, then: the total amounts of phosphorus in the bones show a decrease; the total amounts of phosphorus in the soft tissues increase; the phosphorus concentration of the blood plasma and tissue fluids falls, presumably due to lowered absorption of phosphorus from the gut. We must conclude from these facts, that the soft tissues are able to utilize the phosphorus of the tissue fluids at a level of phosphorus concentration at which deposition of phosphorus cannot take place in the bones. The effect of this difference in efficiency between the soft tissues and the bones is that the phosphorus liberated from the latter by normal bone resorption is now available for the soft tissues. As soon as that level of plasma phosphorus is reached at which no more deposition of phosphorus can take place in the bones, a new supply of phosphorus is made available to the body.

As soon as the mineral balance of the bones becomes negative, not only phosphorus but also calcium is liberated. As the need of the soft tissues for calcium is very small, the circulating fluids will now have an abundant supply of calcium at their disposal. In some of our cases the amounts of calcium liberated from the metatarsals of one foot were about 1 mg. per day, and if the rest of the skeleton loses calcium at about the same rate, it would be a conservative estimate that some 10 to 15 mg. of calcium per day would enter the circulation. As the endogenous calcium losses of an adult man are estimated at less than 100 mg. per day (Bauer, Albright and Aub, '29), the negative calcium balance of the bones would certainly be sufficient to cover the losses by excretion of a rabbit weighing about 500 gm. Even if very little calcium were absorbed, the negative balance of the bones should be sufficient to maintain the calcium concentration of the body fluids at a normal level.

If, in rickets, the absorption of calcium is decreased, then we would expect more severe manifestations of calcium deficiency in mild than in severe rickets. In the latter, the bones would have a negative calcium balance and the requirements of the tissue fluids would thus be filled. In mild rickets, however, with a less severe impairment of the phosphorus absorption, the concentration of plasma phosphorus might, at times, increase to a value where calcification of bones could take place. As soon as that happened, the endogenous supply of calcium would decrease and any deficiency in the absorption of calcium from the intestines would be reflected in a decrease of the calcium concentration of the tissue fluids. This may be the reason why tetany is seen only in cases of mild or healing rickets.

It seems, then, that we do not have to assume the existence of any unknown mechanism in order to explain why, in rickets, the serum phosphorus is low while the serum calcium is normal. The only fact that may need further study is the ability of the tissues to utilize phosphorus from a lower concentration of plasma phosphorus than the bones. This tends to keep the tissue fluids permanently low in phosphorus, and as the low phosphorus makes calcification of bone impossible, all the calcium liberated by the absorption of old bone is now available to maintain the calcium concentration in the tissue fluids at a normal level. Our determinations show that the calcium thus set free ought to be quite sufficient for that purpose, even if the exogenous supply of calcium would be considerably diminished.

SUMMARY

The change in the total amounts of calcium in the metatarsal bones of rabbits was studied during the development of rickets. The method employed was to amputate one leg at the beginning of the experimental period and compare the calcium content of the bones of this leg with that of the other, removed after the end of the period. Suitable controls showed that the calcium content of the bones of one leg was very nearly the

same as that of the other when the legs were removed at the same time; and that in normal animals the amputation of one leg did not prevent the bones of the other leg from growing and increasing in total calcium content.

In seven out of twelve cases the calcium balance of the bones was found to be markedly negative; the amounts of calcium liberated from the metatarsal bones of one leg ranged in these seven cases from 0.45 to 2.10 mg. calcium per day. In the five remaining cases the balance was practically zero.

Even if the amounts of calcium absorbed from the intestines were considerably decreased, the amounts liberated from the bones would, in many cases, be sufficient to maintain a normal concentration of calcium in the tissue fluids.

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AN EASILY CONSTRUCTED RAT METABOLISM APPARATUS WHICH AUTOMATICALLY RE- CORDS OXYGEN CONSUMPTION AND ANIMAL ACTIVITY

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TWO FIGURES

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Uncontrollable activity of the common laboratory animals makes accurate determination of their true, basal respiratory metabolism by any of the usually employed methods almost prohibitively time-consuming; and may even make impossible the securing of necessary records at some critical period of an experimental procedure. This is not so much because they are never in a basal state as because there is no adequate, objective criterion by which it may be known when they are; nor methods sensitive enough to measure accurately such short basal intervals as frequently occur; by the time attainment of basal conditions seems a reasonable guess and an experimental run requiring considerable time for completion is gotten under way, the animal will have moved and it is necessary to begin anew. Nor are attempts to evaluate and thus salvage the less doubtful data by means of an accompanying activity record too helpful; for the usual mechanical registration of activity is, at best, only roughly qualitative; is influenced more by kind than intensity of movement; and there is often most unsatisfactory agreement between the apparent restlessness of the animal and the record of its respiratory metabolism.

In view of these generally conceded difficulties it is surprising that greater effort has not been made to develop methods of objective recording, sufficiently sensitive and accurate to make possible determinations of metabolic rate from basal periods of even short duration. A continuous graphic record of oxygen consumption, or carbon dioxide production, or both, of such sensitivity would, at the same time, automatically eliminate all doubt as to the occurrence or effect of activity; this would be immediately apparent and measurable in its only significant effect, viz., quantitative alteration of the respiratory exchange.

Efforts toward this end have been made; the first one on our part was to secure continuous, graphic records of the changes in weight involved in the use of Haldane's method, as was done independently by Arnoldi and described and used by Asada ('23); in our experience the rubber hose connections between the different parts of the apparatus so interfered with the free movement of the balances as to make the method useless. Next it was attempted to register graphically the movement of spirometers, for oxygen measurement (Hemmingsen, '34; Amano, '35); but our ingenuity was inadequate to devise any type of spirometer sufficiently responsive and sensitive to be of use with animals as small as the rat.

Attention was then turned to adaptation for this purpose of the 'automatic microspirometer' of Hanan ('29); in this ingenious device water from a constant-level reservoir siphons automatically into a burette as rapidly as oxygen is withdrawn from it by the animal; and in its original form it had been successfully applied to the rat metabolism apparatus of Foster and Sundstroem ('26) by J. O. Ralls of this school (unpublished). Although automatic in its operation, this device provided no continuous, graphic record of the volume changes, such as we wanted. Our first attempt to add this refinement was along the line later developed by Lewis and Luck ('33), whereby, upon reaching a certain volume, the displacement water automatically siphons out, record being made of the number of siphonings and thus of the oxygen used.

Though graphic, however, such a record is discontinuous; further, in our experience, the siphonings were never clean-cut and exact and no form of siphon we could devise would make them so. A still further objection to this method as thus employed is that the displacement water is continually renewed and, unless precaution were taken (as Lewis and Luck did) previously to saturate it with oxygen, would absorb indeterminate amounts of this gas as it was brought into contact with it (in the burette) in pure form.

The water displacement method, however, is very sensitive; it involves no mechanical moving parts with friction and inertia; all that is needed is a satisfactory method of recording the displaced volumes graphically and continuously; and, preferably, of operating it as a closed system whereby the same water might be used repeatedly and thus become and remain saturated with the gas (oxygen) with which it is in contact. With this experience and idea in mind the following apparatus was evolved, which among other advantages can be assembled almost entirely from odds and ends obtainable about any laboratory.

DESCRIPTION OF APPARATUS

Figure 1 is entirely diagrammatic; description and dimensions of the essential parts are as follows.

M^1 - M^2 is a water-manometer system of which M^1 is a glass tube, 270 mm. long by 16 mm. diameter, carrying a paraffined, cork float; this float supports a writing point which traces a record (fig. 2) on smoked kymograph paper; the length of M^1 , as given, was so chosen that the maximum excursion of this float and writing point would be from the bottom to top of our kymograph paper during a 30-minute run, i.e., provide a maximum vertical displacement and, hence, accuracy of measurement. M^1 and M^2 are connected with flexible rubber tubing to permit placing M^1 in relation to M^2 so the difference in water level will properly counterbalance the pressure (negative; see below) above the water in M^2 . The connection of M^2 to the rest of the apparatus is also by flexible rubber tubing

(although not so indicated in the diagram) so that M^1 - M^2 may be placed at any convenient position for securing the kymo-graph record, irrespective of the location of the rest of the

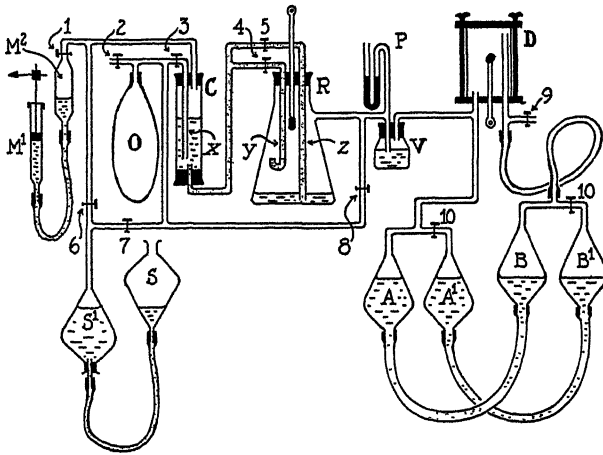


Figure 1

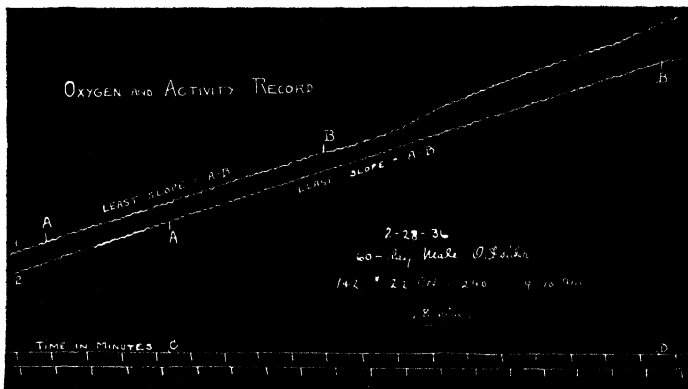


Figure 2

apparatus—as when the latter is in a constant temperature water bath or on another table, etc.

O is a rubber football bladder.

C is a glass cylinder 200 mm. long by 35 mm. diameter, provided at the upper end with a two-hole and below with a one-hole rubber stopper; these dimensions, again, were selected

to meet the particular requirements in mind and would necessarily be altered for use with larger animals, longer runs, etc.

The tube connecting C with R enters the latter by two arms; one of these, Z, extends to the bottom of R and is used (as described later) to draw the water back from R to C; the other, Y, shown with a bent tip, is of such a length that its open end in R is exactly at the same level as the lower end of the tube, X, which extends from the top nearly to the bottom of C; thus ensuring exact equality of pressure at the open ends of X and Y. Since, during operation, oxygen enters C through X from the bag, O, at atmospheric pressure (Mariotte burette principle), water will siphon from C to R through Y in proportionate amount only as the pressure in R tends to be reduced by withdrawal of its oxygen by the animal.

R is a 1-liter suction flask provided with a three-hole rubber stopper to carry a thermometer and the tubes Y and Z just referred to; R might be any size; the suction flask was the most convenient thing at hand for our use.

C and R together contain about 350 cc. of water to which, and to the water in the manometer system M^1 - M^2 , enough CuSO_4 is added to prevent mold growth. This amount of water in C-R is dependent on the size of animals used, length of run desired, etc.

P is an oil manometer for determining changes in pressure in the above system.

V is a 150 cc. wide mouth bottle or flask containing paraffin oil and inserted at this place as a Müller valve to prevent back diffusion and escape from absorption of carbon dioxide from animal chamber D.

D, the animal chamber, is a fruit jar, 190 mm. long by 80 mm. diameter, clamped between two heavy plates; the plate closing the open end of the jar (with a heavy rubber washer) is provided with brass tubes, threaded and soldered into it, for the necessary connections and insertion of a thermometer (in one model the thermometer was completely within the jar attached to the ventilation tube which extends to the bottom of it).

A-B, A'-B' and S-S' are leveling bulbs of 350 cc. capacity with openings at top and bottom of 22 to 25 and 3 to 4 mm., respectively.

A-B and A'-B' are joined by their larger openings with 16 mm. rubber tubing; each pair contains 400 cc., 0.1 N barium hydroxide for absorption of carbon dioxide; for this purpose only one set, A-B or A'-B', is used at a time by appropriate placement of clamps 10; circulation of air from A (or A') through D to B (or B') is obtained by raising and lowering B-B', which are attached (by easily manipulated clips) to the arm of a motor driven crank, making about five revolutions per minute; this provides ventilation of the animal chamber at the rate of about 150 liters per hour.

S-S' contains water.

A cylinder of commercial oxygen (not shown in the diagram) is attached beyond clamp 2 to replenish the supply in O when necessary. Also, before operation, C and R are flushed through and left filled with oxygen.

OPERATION

Oxygen determination

During preliminary periods of adjustment or at any other time when no record is wanted of the oxygen used, all clamps are tightened except 8; this permits oxygen to pass from the bag, O, through 8 and valve V into the animal chamber.

To secure record of the oxygen consumption: the apparatus has been previously set (as described below) so that 1) the water in the system M¹-M² is nearly all in M²; thus, arm M¹ will be nearly empty and the float at its lowest point; 2) C is nearly full of water; 3) all clamps are tightened except 1, 3 and 4, which are loosened.

As the apparatus thus stands (with clamp 1 open) the manometer system M¹-M² will record the pressure above the water column in C; this will be negative (in millimeters of water) by an amount equal to the height of the water level in C above the opening of tube X; tube X connects (through 3, which is open) with oxygen at atmospheric pressure in O; the

open end of X in C is therefore the only point in C at atmospheric pressure.

As the animal uses oxygen, this must come (since clamp 8 is closed) from R; this tends to lower the pressure in R and water will siphon into R from C (clamp 4 is open, 5 is closed) through tube Y. Since, as explained before, the opening of tube Y is at the same level as that of tube X (in C) the pressure at the open end of Y will be the same as that at the open end of X, or atmospheric; thus the pressure of oxygen in R is atmospheric and as such, except for the very slight pressure required to operate valve V, reaches the animal.

As water siphons from C to R the level in C falls, decreasing the height of the column above the opening of X (atmospheric pressure) and thus decreasing the negativity of the pressure in the space above the water; therefore water will run from M^2 to M^1 and the float will rise, giving a record (on smoked kymograph paper) as in figure 2.

The advantage of this method of recording the displacement of water from C to R as a measure of oxygen used is that it permits the recording manometer system, M^1 - M^2 , to be placed at any convenient location irrespective of relation to the rest of the apparatus; thus, with C-R immersed in a water bath for temperature control direct measurement of the water siphoning from C or into R is very inconvenient; and even with C-R accessible, as when the entire apparatus is in air in a constant temperature room, this method of recording permits a flexibility of manipulation that is very desirable. The advantage of a completely closed system over any other we have seen for recording oxygen by water displacement is that the water (in C-R) can be used over and over again, repeatedly (as explained below); and being always in contact with oxygen there is no possibility of error in oxygen measurement due to absorption of indeterminate amounts by the water with which it is in contact.

Capacities and dimensions given are such that for rats of 130 to 230 gm. weight complete emptying of C requires at least $\frac{1}{2}$ hour.

Although any description of the re-setting of the apparatus for another run will sound very cumbersome and time-consuming, actually it can be done with an interruption of only 30 seconds or less between successive periods.

Resetting the apparatus

At the end of an experimental period, as seen above, most of the water of M^1 - M^2 will be in M^1 ; and of C-R in R.

Open clamp 8 so the animal may have uninterrupted supply of oxygen directly from bag, O, during the following manipulations.

Close clamps 3 and 4 and open clamp 5.

Place S lower than S', thus establishing a negative pressure above the water in S'.

Open clamp 6; the negative pressure in S' is thus transmitted in two directions: 1) to M^2 , thus drawing water from M^1 back into M^2 and lowering the float of M^1 to its zero or starting position; 2) to C, thus drawing water back into C from R through tube Z; the water levels in M^2 and C are thus put in position to start a new experimental period. (In practice these two operations are controlled and executed independently by manipulation of clamps 1 and 5.)

Since clamp 8 is open during the above operation, oxygen can fill R from O as water is drawn from R into C; also after closing clamp 6 and opening 7 the oxygen drawn into S' (from C) can be forced back into O (and thus saved) by raising S; clamp 7 is then again closed.

Analysis of the record

Graphic record from which oxygen consumption is measured is shown in figure 2 which illustrates parts of two, consecutive, 30-minute runs. The slope of the curves at any point is determined by the rate of oxygen consumption; basal intervals are clearly evident as those portions of the curves with least slope (sections A-B in both curves); activity on the part of the animal is instantly evident in increased rate of oxygen consumption and a steeper slope of the curve (as beyond B

in the upper curve) and is thus automatically registered as a part of the oxygen record. In any case, vertical differences in heights multiplied by the calibrated volumetric equivalent of the manometer gives the volume of oxygen consumed; reduction to standard conditions ($0^{\circ}\text{C}.$ – 760 mm.) is effected in the usual way.

It is not unusual for a rat to be perfectly quiet throughout a 30-minute run and we have a great many such experimental periods; but the advantage of this type of record is that it permits the selection of basal intervals in a run which would otherwise be wasted or doubtful due to the indeterminate effect of an indeterminate amount of activity and is thus not only a great contribution to accuracy but also vastly conservative of the operator's time.

Determination of carbon dioxide

It is usual to use one set of absorbers, e.g., A–B, for preliminary and intermediate periods for which no record is required. At the beginning of an experimental period, noting the exact time, and as nearly coincident as possible with commencement of the oxygen record (as described above) clamps 10 are changed to the opposite arms of the T's on which they are placed so as to open for use the fresh absorbers, e.g., A'–B'. At the end of the experimental period, again noting the exact time, and as nearly as possible coincident with termination of the oxygen record, clamps 10 are replaced so as to put back into use A–B. A'–B' are removed, set aside for analysis and replaced by a fresh set with which a new experimental period may be begun as soon as the oxygen recorder has been reset and started as described before. Or the preliminary absorbers may be replaced by a fresh set while the experimental period is in progress and a second period begun without any intermission.

Aliquot portions of the barium hydroxide solution are titrated with standardized 0.08 normal HCl using phenolphthalein as indicator, and checked to within 0.02 cc. Precautions are taken to guard against undue exposure of the barium

hydroxide solution while loading the absorbers, taking samples and titrating. It was the practice to allow the barium carbonate to settle out and use only the clear supernatant liquid for the titrations. Truog ('15), Bergman ('25) and Mack ('30) investigating the conventional method of shaking barium hydroxide solution during titration point out that the presence of barium carbonate does not affect the results. Following a similar procedure, samples of barium hydroxide containing barium carbonate were titrated and compared to supernatant samples and were found to check well. However, Martin and Green ('33) investigating this same problem show that carbon dioxide is lost in solutions of barium hydroxide containing barium carbonate, if these solutions are stirred with carbon dioxide-free air and titrated with a standard acid stronger than 0.07 normal. They believe this to be due to the liberation of carbon dioxide from the suspended barium carbonate by local concentrations of the acid. It was therefore deemed advisable to use only the supernatant barium hydroxide solution and thus guard against possible loss of carbon dioxide.

As thus used it is not possible to distinguish between the truly basal and other portions of a $\frac{1}{2}$ -hour run with respect to carbon dioxide production. From the total carbon dioxide and total oxygen of such an experimental period we believe it is possible, however, to derive information of value respecting even the true, basal respiratory quotient. We have always discarded completely any periods in which there was considerable, persistent activity. So far we have analyzed over 400 experimental periods on more than 200 rats that were acceptable in this respect; segregating these into three groups which 1) were strictly basal throughout, 2) showed slight and 3) moderate activity, it has been found that the average respiratory quotient is exactly the same for each, i.e., the amount of activity which we believe is consistent with a basal oxygen determination by this method appears to have no significant effect on the respiratory quotient. This evidence will be published in full later; if true it means that not only the basal

oxygen consumption (which is determined directly) but also the basal carbon dioxide production and respiratory quotient may be determined from the data obtained by this method.

Checking the apparatus

A great amount of time was wasted in the effort to devise an alcohol burner that would simulate the rate of oxygen utilization of a rat. Recourse was finally had to withdrawal of oxygen and introduction of carbon dioxide by means of a burette attached beyond stopcock 9 (fig. 1) at rates comparable to those actually encountered in experimental use. The maximum error in single runs was, for oxygen less than, and for carbon dioxide slightly over 1%; the averages for a large number of such checks showed there was no systematic error in either measurement.

SUMMARY

An apparatus is described for determination of the respiratory metabolism of rats, the significant feature of which is a means of obtaining continuous graphic record of oxygen consumption of sufficient sensitivity to permit selection and accurate measurement of strictly basal intervals of even short duration. This is effected by a modification of Hanan's microspirometer which permits registration of the water siphoning into the oxygen reservoir to replace oxygen as it is used by the animal. Carbon dioxide is measured only for longer intervals of $\frac{1}{2}$ hour or more by absorption and titration in barium hydroxide; the carbon dioxide absorbers serve also as the pumps to keep the air within the closed system in circulation.

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THE EFFECT OF PROLONGED EXPOSURE TO LOW TEMPERATURE ON THE BASAL METABOLISM OF THE RAT

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In connection with work being done in this laboratory on the effect of prolonged exposure to cold on development, estrous cycles and organ weights of the albino rat, the question arose as to what effect, if any, this, also, might have on basal metabolic rate. After the work was well under way it was found that Benedict and MacLeod ('29 b) had reported observations on a few rats kept for 24 hours at 21°C. which indicated that such exposure elevated the basal rate 4.5% above that to be expected if the animals had been previously maintained at 28°C. Also, Ishida ('30), investigating the influence of season, found the basal metabolic rate of the rat at thermal neutrality varied inversely with the previous environmental temperature. According to Hemmingsen ('34) it is important in the evaluation of such results to take into account alterations of body temperature; when this is corrected for, exposure to 20°C. or above for 18 to 24 hours previous to the metabolism measurement, has no appreciable effect on the basal rate. The recent work of Gelineo ('35) on the effect of exposure to high temperatures, while related to the problem in its broad aspects is not immediately relevant and need not be discussed.

PROCEDURE

Having no idea what length exposure might be required to effect maximum change in energy metabolism, it was arbitrarily decided to use periods of 15, 30 and 60 days, during which the experimental animals were kept for the major part of each day in a cold room at average temperatures for the various groups of 7.8 to 12.2°C. (table 1, column 3). Daily, from 8 A.M. to 4 P.M., they were removed to the warm animal room to dry out the cages and, more particularly, because of an early impression that while in the cold they did not eat or drink properly to maintain normal vigor and growth. This was due to preliminary observation that animals kept in the cold failed after a time to gain normally in weight; and it was assumed that if removed to normal temperature for part of the day this might be corrected. As will be shown (see Results: body weight) this was later proved incorrect, but only after so much data had been accumulated under this regime it was deemed best to complete the work as presented here without changing it.

As may be seen from table 1, the major part of the work was done with three groups of fifty female rats. Each group was subdivided into a cold-room unit of twenty-five (II B, III B, IV B) and twenty-five litter-mate controls which were kept in the constant temperature, warm animal room (II A, III A, IV A). In addition, a 60-day group of males (I A and B) was included for sex comparison; these were chosen from the same litters as the 60-day females.

All of the groups were started on the experiment at the same age (65 days) and therefore at nearly the same weight (average, 130 gm.; the 60-day males averaged 5 gm. heavier than their sisters). The metabolism of the 60-day groups was measured in the fall; of the 30- and 15-day groups in the winter. The diet for all was Purina Dog Chow, which we have used for several years and found adequate to maintain growth, vigor and fertility. The animals were kept in wire cages in groups of five. Estrous cycles were followed daily and recorded according to the method of Emery ('31). Careful

TABLE 1

SERIES		1	2	3	4	5	6	7	8	9	10	11
(TWENTY-FIVE ANIMALS EACH)		Av. body weight	Av. rectal temp., °C.	Av. envir. temp., °C.	Av. chamber temp., °C.	R. Q.	cc. O ₂ /gm./hr.	% increase	Cal./sq.m./24 hrs.	% increase	Cal./100 gm./24 hrs.	% increase
I-A	60-day warm-room series (M) ¹	239	36.2	25.5	29.2	0.737	1.06	+15.0	975	+10.1	11.97	+14.8
I-B	60-day cold-room series (M)	220	36.7	7.8	29.1	0.750	1.22		1073		13.74	
II-A	60-day warm-room series (F) ²	170	36.4 ²	25.5	29.0	0.758	1.06	+16.1	881	+12.4	12.15	+16.1
II-B	60-day cold-room series (F)	153	36.3 ²	12.2	29.0	0.766	1.23		990		14.10	
III-A	30-day warm-room series (F)	149	36.6	26.7	28.7	0.761	1.20	+15.0	949	+12.7	13.63	+15.0
III-B	30-day cold-room series (F)	143	37.7	9.5	28.8	0.761	1.38		1070		15.68	
IV-A	15-day warm-room series (F)	134	36.2	26.7	29.1	0.762	1.25	+11.2	969	+11.4	14.52	+11.1
IV-B	15-day cold-room series (F)	134	36.9	9.5	29.2	0.757	1.39		1079		16.13	

¹ (M), males.² (F), females. ³ Represents only a few animals.

record was kept of both warm-room and refrigerator temperatures (table 1, column 3). It may be added that the albino rats of our colony are a hardy, fertile, normal-growing, Wistar strain, inbred for many generations and quite free from internal and external parasitism and organic disease.

Metabolism technic. The animals were fasted 24 hours previous to metabolism determination and during the last several hours of this period were in the warm metabolism room, which was controlled to $\pm 0.5^{\circ}\text{C}$. The animal chamber of the metabolism apparatus was maintained at the critical temperature of the rat, the average for all groups during the determination being 29.0°C . (table 1, column 4) and the range of averages, 28.7° to 29.2°C ., which is in the middle of the critical temperature range of 28° to 30°C .

To induce quiescence, light was shone on the animal chamber; as Horst et al. ('34 b) have shown, this has no effect on basal metabolism; local heating from the lamp, and dead-air around the apparatus was eliminated by use of an electric fan.

Estimation of respiratory metabolism was made with the apparatus previously described (Schwabe and Griffith, '38). At least two 30-minute runs, and more if necessary for unimpeachable basal oxygen determination, were made with each animal. As was explained in connection with description of the apparatus the particular advantage of the continuous, graphic record of oxygen consumption provided by it, is that it makes possible accurate measurement of basal portions of a $\frac{1}{2}$ -hour record which otherwise would be dubious or useless due to sporadic activity. Consequently, as far as accuracy of basal oxygen determination is concerned, the 30-minute runs did not need to be, and the majority were not, strictly basal throughout.

This is important in connection with the determination of carbon dioxide production and necessitates explanation of the respiratory quotients. Carbon dioxide was determined only for the total, $\frac{1}{2}$ -hour run; this, together with the total oxygen for the same period (which was easily computed from the

graphic record) permits calculation of the respiratory quotient. This might be considered the basal value only for those metabolism periods that were strictly so throughout. Early in the work, however, comparison of two consecutive runs, one of which might have been basal and the other with some activity, or of two with different amounts of activity, showed close similarity of quotients. And when the work was completed the more than 400 $\frac{1}{2}$ -hour records were segregated into three classes: 1) those basal throughout, and those showing 2) slight and 3) moderate activity; with the result that the average respiratory quotients of the three groups were identical. This analysis will be published in full later; the result may be used here, however, as indicating that the slight activity during a 30-minute run compatible with a rigidly basal oxygen determination had little or no effect on the respiratory quotient.

Body temperature was measured immediately after the animals were removed from the metabolism chamber with a 1-minute clinical thermometer inserted $1\frac{1}{2}$ inches into the rectum. The animals were then autopsied and body measurements and organ weights determined.

RESULTS

The significant data of the experiment are summarized in table 1; of these the basal metabolic rate interests us most, but before considering it, attention may be given to the effect of the cold exposure on weight, body temperature and respiratory quotient.

Body weight (column 1). Exposure for longer than 15 days inhibits gain in weight; comparing the cold- and warm-room series of each group, the retardation is, for the 60-day males, 9%; 60-day females, 11%; and 30-day females, 4%. This is in spite of the fact, as determined at intervals for limited groups, that the exposed animals ate approximately 30% more food per day than the controls. Since approximately 87% of their food intake was during the night while in the cold room (which compares favorably with the 84% nocturnal ingestion of the controls), and since 8 hours were available during the

day in the warm animal room to supplement any deficiency due to inhibition by the cold, it would appear that failure to gain weight was probably not the result of curtailment of opportunity to eat.

Rectal temperature (column 2). Excluding the 60-day females (II A and B), too few of whom were measured to give reliable averages, it may be seen that exposure to cold results in increased body temperature of 0.5° , 0.7° and 1.1°C. , for the 60-day males, 15- and 30-day females, respectively.

Respiratory quotient (column 5). This appears to be unaffected; the average for all animals is 0.755.

Basal metabolic rate. In order to facilitate comparison with the data reported by others this has been given in the units most frequently used: oxygen per gram weight of rat per hour (column 6); calories per square meter per 24 hours (column 8); and calories per 100 gm. weight per 24 hours (column 10). Computation of body surface was by Diack's formula ('30), and of calories, by the usual method, based on the respiratory quotients of the $\frac{1}{2}$ -hour runs, as explained above.

Exposure to cold increases basal metabolic rate. The increase per unit of body weight is identical in terms either of oxygen consumption, as actually determined (column 7), or of the calculated heat production (column 11), with a maximum of 15 to 16%, which is attained sometime between the fifteenth and thirtieth day of exposure and is maintained without change at least as long as the sixtieth day.

Per unit of body surface the increase (column 9) is the same as per unit of weight up to the fifteenth day; thereafter, it is 2.3 and 3.7% less for the 30- and 60-day females, respectively; and approximately 4.7% less for the 60-day males. This is undoubtedly a result of the deleterious effect of cold exposure on gain in weight which was referred to earlier; and it is a nice problem, but one which it would be fruitless to pursue here, as to which method of expressing the results is physiologically preferable.

In any terms, this increased metabolic rate, as determined on this limited sample is statistically significant. In order to test this most severely, analysis has been made of the data per unit of surface, which, as just seen, gives the lesser differences. In this unit, the mean differences divided by their standard errors give values of 5.8, 5.1, 4.4 and 4.9 for the 15-, 30- and 60-day females, and 60-day males, respectively (table 2). Since a difference which is three or more times its standard error has validity, it may be concluded we are dealing

TABLE 2

Formulae used: $\frac{S.D.}{\sqrt{n}} = S.E.$ $\sqrt{(S.E.^2) + (S.E.^2)} = S.E. \text{ of difference.}$

ANIMAL SERIES	RANGE, CAL./M. ² /24 HRS.	MEAN \pm S.E.	$\frac{\text{MEAN DIFF.}}{\text{S.E. DIFF.}}$
60-day warm-room (M)	820-1110	975 \pm 16	4.9
60-day cold-room (M)	930-1230	1074 \pm 17	
60-day warm-room (F)	720-1040	881 \pm 18	4.4
60-day cold-room (F)	760-1150	990 \pm 21	
30-day warm-room (F)	860-1130	949 \pm 13	5.1
30-day cold-room (F)	900-1300	1070 \pm 18	
15-day warm-room (F)	840-1130	969 \pm 14	5.8
15-day cold-room (F)	910-1170	1079 \pm 10	

here with a real effect. It may be important that the greatest significance, according to this test, attaches to the effect produced within the first 15 days. Here, again, the later results may be vitiated by the retarded gain in weight which is characteristic of the longer exposures.

DISCUSSION

Body temperature. The increased metabolic rate of the animals kept in the cold cannot be attributed solely to higher body temperatures since Hemmingsen's ('34) correction still leaves differences of 8 to 12% between the warm- and cold-room series of the various groups.

The average rectal temperature of both warm- ($36.4^{\circ}\text{C}.$) and cold-room ($37.1^{\circ}\text{C}.$) series is somewhat lower than that given in Donaldson's tables ('24) and reported by Benedict et al. ('32) and Horst et al. ('34 a). It has been pointed out that the rat is not strictly a homothermal animal; thus it is conceivable that the environmental temperature previous to the metabolism determination as well as the room temperature at which the body temperature is measured could appreciably affect the result; furthermore, the method of taking the temperature of such an animal as the rat is of importance, varies considerably and is not always stated. Because of these factors a discussion of the relation of body temperature to basal metabolism on the basis of this and other reported work seems inadvisable.

Age and sex. There is previous evidence (Hill and Hill, '13; Mitchell and Carman, '26; Houssay and Artundo, '29; Horst, Mendel and Benedict, '34 b, c; Landelius and Ljungkvist, '34; Davis and Hastings, '34) that the energy metabolism of the rat decreases with advancing age both per unit of weight and of surface and this is borne out by the data in columns 8 and 10 for both warm- and cold-room females; the slight difference between the 30- and 15-day groups is insignificant unless Hemmingsen's ('34) correction for body temperature is applied, when it, also, becomes appreciable.

Also in agreement with previous work (Mitchell and Carman, '26; Benedict and MacLeod, '29 b; and others), the metabolic rate of the 60-day males is approximately 10% greater than that of their litter-mate sisters per unit of body surface (column 8); this is true whether one compares the warm-room controls (I-A vs. II-A) or the exposed animals (I-B vs. II-B). On the other hand, per unit of body weight, the difference is negligible (I and II A, 1.5%; I and II B, 2.6%; column 10).

The very similar difference in weight between the warm- and cold-room males (I A and B, 19 gm.) and the warm- and cold-room females (II A and B, 17 gm.) together with the fact that the males were exposed to an environmental temperature

4.4°C. lower than the females, suggests that the male rat may be better able to withstand cold than the female. This might account for some of the apparent sex differences noted.

Amount of exposure. With the exception of the 15-day group, the rats exposed to low temperature did not gain in weight as rapidly as their litter-mates kept in a constant warm environment; exposure for longer than 15 days is deleterious.

Maximum increase in energy metabolism on the part of animals exposed to cold is attained by the thirtieth and sustained up to the sixtieth day. It would be of interest to know just when the maximum increase is reached; and, also, how much longer it would be maintained at this maximum level. Horst et al. ('34 c) have shown that slow-growing and stunted rats have a lower metabolic rate than rapidly-growing and normal-size animals. Since the cold-room animals do not maintain a normal growth but actually grow less and less rapidly as their stay in the cold is prolonged it will be of interest in future work to discover whether a time would be reached or body weight prevail when the enhanced rate declines; or whether the increased level will be maintained although the animals are progressively stunted in growth.

Respiratory quotient. The respiratory quotient of the fasting rat is at present such a controversial issue we do not expect acceptance of our figures as absolute, basal values without more support and defense than is in place here; this is planned for a later report. Our only concern here is whether the change in metabolic rate produced by cold was accompanied by qualitative changes in metabolism. And since all determinations were made under the same rigidly controlled conditions we can see no reason to doubt that significant differences in respiratory quotient would have been detected had there been any. We believe the evidence may be accepted as conclusive that exposure to cold had no effect on the nature of the foodstuffs metabolized by the fasting rat in spite of the considerable elevation of metabolic rate.

Surface area and body weight relationship. The use of the Diack ('30) formula for this investigation was largely a matter of personal preference; although, as Diack points out, it

gives results more nearly in line with those for other animals and man. Considering only the 30- and 15-day warm-room, female controls, their average caloric output is 959 Cal./m.²/24 hours. This figure is in agreement with that of others (Benedict and MacLeod, '29 a, b; Verzar, '29; Hari, '24; Goto, '23) when the Rubner formula which they used is applied to our data. The heat production of the male controls is slightly higher than Benedict and MacLeod ('29 b) report for male rats.

Cause of the increased basal metabolic rate. The cause of this increase in basal metabolic rate as a result of exposure to low temperature is not clear. Krogh ('16) has suggested that the increased muscular activity of an animal exposed to cold may be a factor increasing muscle tonus at rest and so affect the basal rate; presumably such an effect might last for some time and thus be a factor in the increased rate which this work reveals.

The work of Hildebrandt ('21) indicates that it is not due to thyroid regulation, because when that gland is removed and the animal exposed to a cold environment during its metabolism measurement, or as Ring ('36) recently reported, previous to its basal determination, the same difference prevails. Leites ('35) speaks of an autoregulation of metabolism—metabolites that take part in the regulation of metabolism similarly to endocrine products; but this is too indefinite to demand attention here except to indicate the, as yet, unknown cause of the effect we have observed and measured.

SUMMARY

Exposure of the albino rat to low temperatures (7 to 12°C.) for the major portion of each day, for 15-, 30- or 60-day periods: 1) increases basal metabolic rate as measured at thermal neutrality, 11 to 16%; this increase, per unit of weight, reaches a maximum and sustained level between the fifteenth and thirtieth day; per unit of body surface the effect is slightly less, though still statistically significant; 2) increases body temperature; correction for this still leaves a

significant increase in metabolic rate; 3) retards gain in weight; 4) does not affect the respiratory quotient.

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THE EFFECT OF THE SULPHYDRYL COMPOUNDS ON MILK PRODUCTION¹

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TWO FIGURES

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Daggs and Tomboulion ('35) have shown the stimulating effect of various dietary substances on milk production in the normal albino rat. The dietary principle that augmented normal lactation in rats receiving the necessary vitamins and minerals was limited to the protein fraction. The fact that the principle was not lost upon autolyzing liver showed it to be associated with protein degradation products. The protein degradation products, extracts and amino acids that contained the lactation principle all had a relatively high sulphur content. That this sulphur had to be organic was indicated by the fact that potassium sulphide had no effect whereas cystine had a very marked effect. It was their belief that the sulphydryl compounds in some way afforded the active principle in augmenting milk production by dietary means. The present study involves further proof of that belief.

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² Submitted by Miss Lidfeldt as partial fulfillment of the requirements for the master of science degree, University of Rochester.

The method used was the same as that reported by Daggs ('35). The rats were maintained on a stock diet of Purina Chow until a few days before parturition when they were placed in special cages and given experimental diets. All litters were limited to six young which were weighed en mass every day. Only 'perfect' litters were used in calculating the results. 'Perfect' litters refer to experiments where the mother has shown no signs of ill health and has successfully cared for and weaned all six young allotted to her at parturition. The lactation indices were calculated from the slopes of the logarithmic growth curves of the suckling young. K_1 is the slope of the logarithmic growth curve from the fourth to the tenth day and K_2 that of the curve from the tenth to the seventeenth day after birth. The addition of K_1 and K_2 , disregarding the decimal point, gives the 'lactation index'—merely a numerical means for comparing results. (See the above quoted papers for the details of the method and the care of the animals.)

Table 1 gives a composite of the diets used in the present study. They were fed ad lib. with the exception of the daily supplements.

The results obtained by the use of the various diets are listed in table 2. The diets are all designated by their sources of protein, amino acids, etc. The other constituents are essentially the same in all diets unless otherwise indicated. The protein level was kept at a minimum so as to determine better the difference between various protein sources since a high protein diet in itself augments lactation. Casein (15) was used as a basal diet, the (15) referring to the parts of casein in the diet. Comparisons were made to this basal ration which gives a lactation index of 760.

Daggs and Tomboulion ('35) cited three experiments on a mixture of cystine, glycine and glutamic acid. (It is known that the body is capable of making glutathione from these three amino acids.) From these few experiments the indication was that the simulated glutathione diet was superior to the cystine diet. When more experiments on this diet were

TABLE 1
Composition of diets

DIETS	CASEIN (15)	CYSTEINE	CYSTEINE GLUTAMIC	METHIONINE	CYSTEINE	CYSTEINE GLYCINE GLUTAMIC	LYSINE	TAURINE	Na TAURO- CHOLATE	CYSTEINE (2) CHOLINE (1)	CYSTEINE (5)	GLYCINE	GLUTAMIC	CYSTEINE (2)	S-BENZYL- CYSTEINE
Casein	15	14	12	14	14	14	12	14	12	12	10	14	14	13	14
Salt mixture ¹	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Agar agar	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Starch	76	76	76	76	76	76	76	76	76	76	76	76	76	76	76
Lard	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
l-cystine		1	1							2	5			2	
Cysteine-HCl					1	1						1			
Glycine			1			1							1		
d-glutamic acid			1			1									
dl-lysine-HCl							1								
dl-methionine				1											
Taurine								1							
Na taurocholate									3						
Choline-HCl										1					
S-benzyl-cysteine ²															1

Wheat germ oil, 5 drops per rat per day.

Cod liver oil, 6 drops per rat per day.

Yeast (dried), 3 gm. per rat per day.

¹ Modified Osborne and Mendel salt mixture (see Daggs, '35).

² Made by Dr. C. E. Braun, department of organic chemistry, University of Vermont.

completed, the average lactation index was slightly lowered so that it seemed probable cystine was just as effective as the cystine, glycine, glutamic acid mixture. This is a reasonable assumption since the body can easily supply the glycine and the glutamic acid but not the cystine. This finding indicated that the S-H group was the essential factor. To substantiate this claim various S-H compounds were tried as well as the separate amino acid constituents of glutathione. In order to be sure that the cleavage of cystine to cysteine by the body did not hamper the results the three amino acids composing

TABLE 2
Lactation indices of sulfur-containing compounds

	K ₁	K ₂	LACTATION INDEX	NUMBER OF LITERS
Casein (15)	0.0488	0.0272	760	9
Cystine glycine glutamic	0.0588	0.0343	931	18
Cystine	0.0586	0.0350	936	6
Glycine	0.0510	0.0260	770	7
Glutamic	0.0504	0.0244	748	9
Cysteine glycine glutamic	0.0595	0.0337	932	11
Cysteine	0.0579	0.0363	942	10
Methionine	0.0577	0.0358	935	8
Lysine	0.0475	0.0246	721	4
Cystine (2)	0.0550	0.0340	890	13
Cystine (5)	0.0475	0.0253	728	7
Taurine	0.0565	0.0258	823	10
Cystine (2) choline (1)	0.0560	0.0333	893	9
S-benzyl-cysteine	0.0460	0.0238	698	5

glutathione, namely cysteine, glycine and glutamic acid, were tried together and singly. The lactation index (932) for the cysteine, glycine, glutamic acid mixture was identical with that (931) found for cystine, glycine and glutamic acid. Cystine, index 936, is just as effective as cysteine, index 942. Glycine and glutamic acid showed no increase over the basal giving indices of 770 and 748, respectively. These facts seem to prove the point that the S-H group is the essential factor. Since methionine is capable of replacing cystine in growth studies it was assumed that it might play a similar role in

these studies. Methionine proved to be equally as efficient as cysteine giving indices of 935 and 942, respectively.

It has been argued that the better rate of growth shown by young suckling a cystine fed mother was due to the cystine passing directly into the milk thus having its effect upon the growth processes in the young. To further substantiate the negative results obtained by feeding cystine directly to the suckling young (Daggs and Tomboulion, '35, p. 590) lysine, another growth stimulating amino acid, was fed to lactating rats. Lysine showed an index of 721 proving its ineffectiveness in stimulating lactation under the conditions of this experiment. More detailed experiments on the relation of lysine to lactation are in progress. When poorly lactating human mothers were fed cystine their milk, when analyzed by Okuda's method, showed no greater amounts of the S-H group than did human milk taken from mothers not fed cystine. This again substantiates the claim that the S-H group does not act by passing into the milk and affecting the growth of the young directly.

The next step was to obtain some idea of the extent to which the cystine content of the diet could be raised before becoming ineffective or toxic. When 2 parts of cystine (cystine (2) table 2) were used very little effect was lost whereas 5 parts of cystine (cystine (5)) did prove quite ineffective (index 728). One part of cystine in the diet is evidently optimal.

It was desirable to try to gain some knowledge of the action of the S-H group in stimulating lactation. The first step in the catabolism of cystine is its reduction to cysteine. This easily takes place within the body making available the S-H group. The next step is thought to be an oxidative deamination, the ammonia going to urea and the sulphur eliminated mostly as inorganic sulphate. However, some of the sulphur may be eliminated in the neutral sulphur fraction. Taurine is thought by some to be an intermediate in the metabolism of cysteine. That this reaction occurs in the body is not established. Lewis ('35) has suggested that the formation of taurocholic acid in the body occurs subsequent to the conjugation of cholic acid with cystine. Taurine is oxidized with

difficulty if at all (Schmidt and Clark, '22). The fact that taurine cannot replace cystine for growth has been shown by many. Even though these findings point to the fact that taurine probably is not directly concerned in the oxidation of cysteine in the body it was tried in these lactation studies because the sulphur mechanism concerned in lactation may not be the same as that for growth. It will be remembered that taurine definitely cannot replace cystine for growth. Taurine gave a lactation index of 823 showing that it did augment lactation some but not nearly so well as the S-H compounds. Since the place of taurine in the intermediary metabolism of cysteine is not well established, it is difficult to explain its function in lactation.

Lewis ('35) points out that when S-substituted derivatives of cysteine are used, thus eliminating the presence of a free S-H group, oxidation in the body does not occur. S-benzyl-cysteine is a compound wherein the sulphur is held in a still different combination than either that of methionine (S-CH₃) or taurine (SO₃H). S-benzyl-cysteine when fed to lactating rats gave an index of 698 showing it to be definitely inactive in augmenting lactation.

Bennett ('37) quotes Hammett as postulating that an increase in the state of oxidation of S-H means a decrease in its reversible reduction capacity. Bennett ('37) has found that l-cystinedisulphoxide (-SO-SO-) will replace cystine for growth, but l-cysteinesulphinic acid (-SO₂H) will not, thus partially confirming Hammett's prediction. When S-(guanylthio)-cysteine-2HCl (-SOH) was used some replacement occurred. This is explained by Bennett as probably due to the cystine formed from S-(guanylthio)-cysteine-2HCl since it is theoretically capable of yielding 33 to 50% cystine. Taurine is partially utilizable for lactation but not for growth. Therefore, it appears that the S-H derivative necessary for lactation can be of a higher oxidized nature than that necessary for growth. Before this can be definitely settled other derivatives similar to the compounds Bennett used should be tried in lactation studies.

The statement made by Lewis ('35) concerning S-H compounds and growth, i.e.,

the peculiar nutritional role of the three sulphur containing amino acids which appear to be interchangeable in growth promotion is not simply a function of the disulphide (S-S) or sulphydryl (S-H) group alone, but is related to the structure of the molecule as a whole. Only the S homologues containing an alpha amino group and three or four carbon atoms appear to possess significant biological properties



Fig. 1 Section of liver from a lactating rat fed the cystine (2) diet.

does not appear to hold entirely for the sulphur containing compounds stimulating lactation unless the rat is capable of converting some taurine back into cysteine. This, however, seems highly improbable (Muldoon, Shipley and Sherwin, '24).

If taurine stimulates lactation perhaps taurocholic acid or sodium taurocholate would retain some lactation stimulating effect. When sodium taurocholate was tried it proved a failure. Therefore in the conjugation of taurine with cholic acid to form taurocholic acid the available lactogenic property of taurine is destroyed.

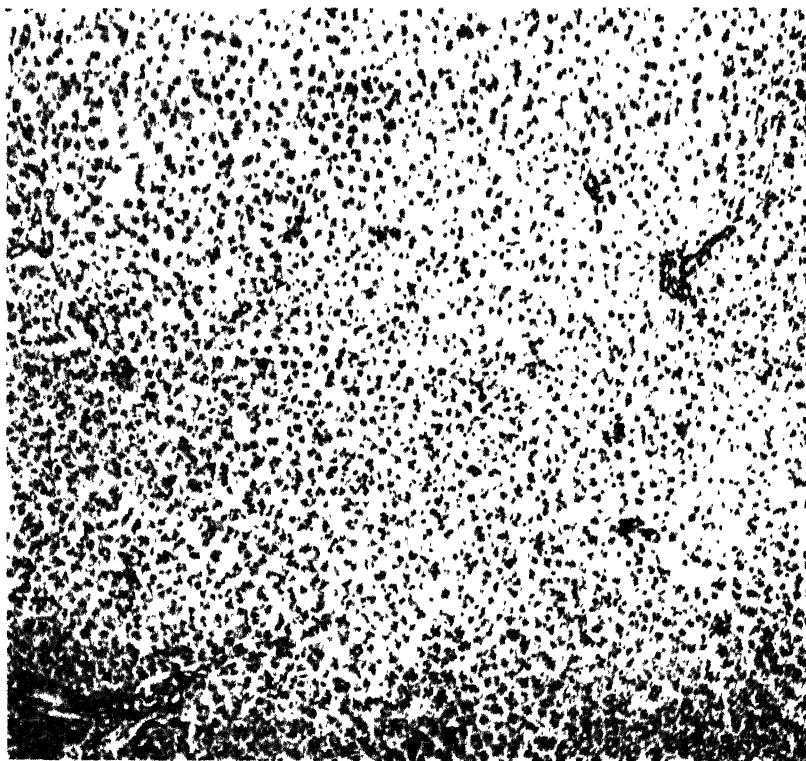


Fig. 2 Section of liver from a lactating rat fed the cystine (2) choline (1) diet.

It was noticed that the livers of the animals fed cystine (2) and cystine (5) diets appeared enlarged and milky. Knowing of the work of Beeston and Channon ('36) it was assumed that these livers would show a marked fatty infiltration on histological examination. This proved to be the case. Figure 1 shows a liver from an animal fed cystine (2). Several

facts have indicated that the liver is probably involved in the lactation process. It is the best natural protein source of the lactation principle (Daggs, '31). Mapson ('32) has shown it to contain a lactagogue and Nakahara, Inukai and Kato ('34) claim to have extracted a potent lactagogue called 'factor L.' Since cystine stimulates lactation and also causes fatty changes in the liver perhaps these changes are essential to the lactation stimulating mechanism. Best and Channon ('35) have shown that choline will prevent fatty livers. When 1 part of choline was fed along with cystine (cystine (2), choline (1)) the lactation index was 893, identical with that for cystine (2), (890), showing that the prevention of the fatty liver did not limit the lactation stimulating effect of cystine. Figure 2 shows a section of liver from a rat on cystine (2) choline (1).

Several animals on each diet were killed at the end of the seventeenth day following parturition. The following tissues were examined histologically: liver, kidney, adrenal, pituitary and mammary gland. With the exception of the aforementioned changes in the liver no abnormalities could be detected. Although many of the mammary glands from cystine (2) fed animals showed a greater number of proliferating follicles and fewer resting alveoli even at the periphery than normals, it did not seem markedly significant since sections from glands of rats on stock ration showed wide variations in appearance.

CONCLUSIONS

1. The sulphydryl amino acids existing either as such (cysteine) or in the potential form (cystine, methionine), are dietary lactagogues.
2. The simulated glutathione, that is, both the cystine, glycine and glutamic acid diet and the cysteine, glycine and glutamic acid diet, each acts as a lactagogue paralleling the effect of cystine or cysteine alone.
3. The non-sulphur amino acids of the tripeptide glutathione, glycine and glutamic acid, have no lactogenic action.

4. In the intermediary metabolism of the sulphydryl compounds, the lactogenic effect is not entirely destroyed at the taurine stage.

5. The catabolic pathway of taurine to form taurocholic acid in the process of milk production is improbable.

6. It is not possible to surpass the animal's hereditary capacity for lactation by increasing the level of the sulphur amino acid content of the diet.

7. It is very improbable that the effect of the sulphydryl group is through the liver, for there is no relationship between fatty infiltration of the liver and milk production.

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THE PARALYSIS IN THE YOUNG OF VITAMIN E DEFICIENT FEMALE RATS ¹

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ONE PLATE (FOUR FIGURES)

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A specific nutritional disease in the young of female rats on vitamin E deficient diets was first described by Evans and Burr ('28). On or about the twentieth day of life the young animals developed a paralysis, especially of the hind quarters. Many of the afflicted rats died, some recovered completely, and a few lived but remained partially paralyzed through life. These investigators showed that the early administration of vitamin E concentrates to the young or to their mothers prevented the onset of the symptoms.

Some anatomical and histological changes in the nerves of such paralyzed animals have been described by Lipshutz ('36). He observed degenerated nerve tracts in the cord which he considered responsible for the paralysis.

The possibility that there might be some relationship between this disease and the muscular lesions which appear in guinea pigs and other Herbivora on purified diets (Goettsch and Pappenheimer, '31; Woodward and McCay, '32; Madsen, McCay and Maynard, '33; Victor, '34) suggested a histological study of the muscles of the paralyzed rats. The nervous systems of the same animals have also been carefully examined.

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EXPERIMENTAL

The basic vitamin E deficient diet used contained:

	%
Casein	18
Sucrose	45.5
Lard	22
Yeast ³	8
Salts ⁴	4.5
Cod liver oil	2

It is adequate for growth and, with vitamin E supplementation, for reproduction, but not for lactation except in a few cases (approximately 5%) even though dry yeast is offered ad libitum to the lactating animals. Of the litters which did survive, less than half developed the paralytic disease.

Young rats developing the paralytic symptoms were carefully watched. When the condition became so acute that death was expected within 12 hours (23 to 36 days of age), they were killed and the skeletal muscles and nerves were removed for histological study. Figures 1 to 3 are photomicrographs of sections from the leg muscles of paralyzed rats. It is readily apparent that the muscles are badly damaged. There is a marked proliferation of the nuclei and necrosis of the muscle fiber (fig. 1). In some cases hyaline necrosis of the fibers could be observed (fig. 2). Accompanying these changes at times was a severe cellulitis with numbers of inflammatory cells scattered through the inflamed area (fig. 3). Such muscle damage in greater or less degree has been observed in every animal which showed any external symptoms of leg weakness or paralysis. Figure 4 is a section of the muscle from a normal rat 22 days old.

Skeletal muscles from various sections of the body all showed the same changes. Histologically, at least, the heart muscle was not affected. As a control experiment, a part of one of the sciatic nerves was removed from eighteen rats at varying ages from 1 to 20 days. The muscles of these animals were then examined at 23 or 26 days of age. The

³ Courtesy of Northwestern Yeast Company.

⁴ Hawk and Oser ('31), *Science*, vol. 74, p. 369.

sections demonstrated the atrophic fibers and nuclear proliferation characteristic of this type of lesion but in no case did they show the marked necrosis of individual fibers, cellulitis, etc., which characterized the muscles of vitamin E deficient young. Contrary to Lipshutz's observations, no abnormalities in the nerves, cord, or brain could be observed.⁵

DISCUSSION

The sudden occurrence of the paralysis in second generation rats in a very specific age group is a baffling phenomenon. Sections from muscles of first generation animals, fed vitamin E deficient diets from weaning and for several months (Olcott and Mattill, '37) showed no degenerate fibers.⁶ On the other hand, second generation animals which escaped the paralytic symptoms (presumably because their stores of vitamin E were sufficient to protect them through the critical period), never developed symptoms later in life although they were maintained on the vitamin E-deficient regime. Sections of muscles obtained from these rats showed occasional necrotic fibers.

The paralysis resembles that observed by Pappenheimer and Goettsch and others in guinea pigs and rabbits in that the lesions are primarily muscular and do not involve the nervous system (Rogers, Pappenheimer and Goettsch, '31; Victor, '34). The histopathological condition of the muscles is also strikingly similar.⁷

Goettsch and Pappenheimer ('31) showed that vitamin E concentrates alone did not prevent and could not cure the so-called nutritional muscular dystrophy in guinea pigs. Nevertheless, all of the effective foodstuffs which they investigated contained vitamin E. Morgulis and Spencer ('36)

⁵ Nissl, Marchi and haematoxylin-eosin stains were used in examining the nervous systems.

⁶ We were not able to duplicate the results of Ringsted ('35) whose rats became paralyzed after 18 to 22 weeks on a vitamin-E deficient ration. Burr, Brown and Mosely ('37) have recently demonstrated paralytic symptoms in rats after 22 months on a diet in which vitamin E was not supplied.

⁷ Comparison made with sections from the muscles of guinea pigs fed Pappenheimer and Goettsch's diet 13 by Prof. H. M. Hines of the department of physiology.

have recently demonstrated that at least two independent factors are required. The analogous pathological picture seen in rats suggests, as do Morgulis and Spencer, that one of these factors may be vitamin E.

If this hypothesis is correct, it would furnish a possible explanation for results obtained by Madsen ('36), who showed that the inclusion of cod liver oil in diets fed to *Herbivora* markedly increased the incidence and severity of the disease. The oxidative reactions initiated by the autoxidation of cod liver oil are destructive to vitamin E (Cummings and Mattill, '31) and the diets containing cod liver oil possibly had much less of this vitamin than those from which cod liver oil was omitted.

I am grateful to Dr. H. P. Smith and his staff in the department of pathology and to Dr. C. G. Barer of the department of neurology for their help in the preparation and interpretation of the tissue sections.

SUMMARY

The paralysis in the young of vitamin E deficient female rats is due to lesions of the skeletal muscle very similar in character to those observable in the so-called nutritional muscular dystrophy of *Herbivora*.

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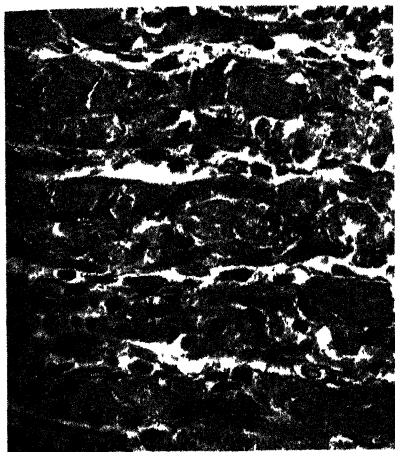
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PLATE 1

EXPLANATION OF FIGURES

- 1 to 3 Sections of gastrocnemius muscles from paralyzed animals.
4 Section of normal rat gastrocnemius muscle. Slides stained with hematoxylin and eosin. $\times 200$.



THE EFFECTS OF SMALL AMOUNTS OF ETHYL ALCOHOL ON THE RESPIRATORY METABOLISM OF HUMAN SUBJECTS DURING REST AND WORK

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Our knowledge of the part that ethyl alcohol is capable of playing in normal metabolic processes has been considerably clarified by the work of Mitchell ('35). He has shown that when ethyl alcohol is added to a complete diet growth is accelerated and the retention of both nitrogen and fat increased. He concludes that "the energy of ethyl alcohol is to a large extent available for physiological purposes." Mitchell's work, however, gives no direct evidence as to the effect of muscular exercise on the metabolism of alcohol. The earlier investigators, notably Atwater and Benedict ('02), Mellanby ('19) and Sommerkamp ('24), concluded, as a result of their investigations, that the energy of alcohol was available for the performance of muscular work. On the other hand the more recent investigations of Carpenter, Lee and Burdett ('33), Canzanelli, Guild and Rapport ('34) and Nyman and Palmlov ('34) seem to show that muscular exercise has no effect upon the rate of oxidation of ethyl alcohol. It should be pointed out, however, that Carpenter, Lee and Burdett used but one human subject and that the conclusions of Canzanelli, Guild and Rapport are based on observations made on a single dog. Furthermore, from a theoretical aspect such conclusions are difficult to explain in view of the fact that alcohol seems to be present normally in body tissues (Gettler, Niederl and Benedetti-Pichler, '32), and that it is

usually postulated as an intermediary product in the normal metabolism of carbohydrates (Grafe, '33). Mitchell's demonstration of the ability of ethyl alcohol to produce growth would seem to justify the conclusion that alcohol could be used at least indirectly in the performing of muscular work since he has proved that a part of its energy can be stored in the animal organism. Carpenter ('33) has reviewed this subject and concludes "that the literature by no means furnishes adequate data to draw a decisive conclusion in regard to whether or no muscular activity accelerates the combustion of alcohol."

METHODS

We have investigated the effects of small amounts of ethyl alcohol on the respiratory exchanges during rest and work. Two series of experiments have been carried out using five different subjects upon whom a total of more than fifty tests were run. The oxygen consumption, carbon dioxide production and total respiratory volume were determined by means of the Tissot-Haldane technic. Expired air was collected in the Collins chain compensated gasometer. For basal and resting tests spirometers of 100 liters capacity were used while in the work tests a double chain compensated gasometer of 700 liters capacity was used. Samples of expired air were analyzed in the Boothby modification of the Haldane gas analysis apparatus (Boothby and Sandiford, '20). This apparatus was checked by frequent analyses of outdoor air. For the work test a bicycle ergometer of the Prony brake type (manufactured by Warren E. Collins) was used.

In the first series of experiments three subjects were used. With these subjects both control and alcohol tests were run while the subject was lying in a basal condition, while he was sitting at rest on the bicycle, and while he was pedalling the bicycle at a rate which was equal to 2500 to 3000 foot pounds of work per minute. Following the work period the expired air was collected during a recovery period of 35 minutes.

The arrangement of the spirometers was such that the collection of expired air was continuous during work and recovery, none of it being allowed to escape. In basal tests the procedure was as follows: the subject reported to the laboratory in a post-absorptive condition and rested on a comfortable bed for the usual $\frac{1}{2}$ -hour period. One or two basal metabolism determinations were then made. The subject then ingested 200 cc. of water if the test was a control test, or 200 cc. of approximately 10% alcohol in alcohol tests. (The quantity of alcohol given was from 0.25 to 0.30 gm. per kg. of body weight). The respiratory metabolism was then determined at $\frac{1}{2}$ -hour intervals for approximately 2 hours.

In the work tests the subject came to the laboratory in a post-absorptive condition and ingested the water or alcohol solution according as the determination was to be a control or alcohol test. The dosage of alcohol was the same as that used in basal tests. The subject then sat quietly on the bicycle ergometer for 15 to 20 minutes after which two determinations of the respiratory exchanges during rest were made. The values obtained in these tests were used as a base line from which the excess metabolism of work was calculated. After the usual washout periods the respiratory exchanges were measured for a 10-minute work period and a 35-minute recovery period.

In the second series of experiments no basal tests were run. Approximately the same quantity of alcohol was ingested but in this case it was diluted with 200 cc. of a 10% dextrose solution. Control tests were run in this series 1) in which 200 cc. of water was ingested and 2) in which 200 cc. of 10% dextrose was given. It seemed desirable to test the effects of alcohol plus dextrose upon the respiratory exchanges during rest and work since the recent work of Carpenter and Lee ('37) indicate that the metabolism of alcohol is considerably accelerated if it is ingested with glucose. Haggard and Greenburg ('37) have also shown recently that the toxic effects of alcohol are greatly decreased if the blood sugar is elevated. Both of these investigations seems to indicate

an intimate relationship between the metabolism of ethyl alcohol and that of dextrose. In all other respects the experiments in this second series were similar to those of the first series. Three subjects were used in this series of experiments but one of these had already served in the first series and therefore the total number of subjects was five.

RESULTS AND DISCUSSION

The results of the experiments on basal metabolism carried out in the first series are shown in table 1. In tabulating our results we have shown only averages. The degree of variation between individual determinations has been indicated by the standard deviation which in each case follows the tabulated average under the heading SD. This makes it a simple matter to compare the degree of variability obtained in individual tests with the average difference between control and alcohol tests. The oxygen consumption was apparently affected by the ingestion of alcohol only slightly, if at all. With two of the subjects there is no significant change while in the third subject there was an increase of about 6%. However, with this subject (JRM) there was but one basal test preceding the administration of alcohol which makes this apparent rise of little significance. This is all the more true in view of the fact that the oxygen consumption during control tests is approximately the same as that following the ingestion of alcohol.

All three subjects show a noticeable drop in the R.Q. following the ingestion of alcohol. This is 0.04 in the case of FAH. The variability obtained in the tests following alcohol is considerably less than that of any other series in the case of both subjects FAH and JRM. The drop of 0.02 which occurred in the R.Q. of subject RCG following the ingestion of alcohol is of doubtful significance since in control tests a spontaneous drop even greater than this occurred. The results obtained on FAH may justly be considered of greater significance than those on RCG and JRM since the total number of tests on him was about twice as great as on

TABLE I
The effect of alcohol on the basal oxygen consumption respiratory quotient and ventilation equivalent

SUBJECT	TESTS		BEFORE						Number of tests	AFTER				
	Type	Number	Cubic centimeters O ₂ per minute	SD.	R.Q.	SD.	Ventilation equivalent O ₂ ¹	SD.		Cubic centimeters O ₂ per minute	SD.	R.Q.	SD.	Ventilation equivalent O ₂ ¹
FAH	C.	8	264.6	1.8	0.82	0.001	1.92	0.03	260.6	2.8	0.81	0.001	2.04	0.03
	A.	7	266.0	2.8	0.83	0.001	1.89	0.02	265.4	1.3	0.79	0.003	1.99	0.02
ROG	C.	4	186.5	5.2	0.82	0.016	1.88	0.03	188.6	2.2	0.79	0.011	1.92	0.03
	A.	3	188.2	3.4	0.82	0.014	1.93	0.01	187.6	1.7	0.80	0.012	1.96	0.02
JRM	C.	4	230.5	1.1	0.79	0.012	2.24	0.10	236.0	1.3	0.81	0.016	2.26	0.09
	A.	1	214.5		0.84		2.12		227.1	2.0	0.82	0.006	2.27	0.04

¹Liters of air exhaled per 100 cc. of oxygen absorbed.

either of the other two subjects. If with this subject we take the drop in the quotient as a measure of the amount of alcohol being oxidized, we calculate that during the 2 hours immediately following the taking of the alcohol this substance was oxidized at the rate of about 30 mg. per minute.

In any study of respiratory exchanges a knowledge of the ventilation volume is of major importance since it is generally agreed that fluctuations in the total quantity of air breathed have a marked effect upon the excretion of carbon dioxide. Recording the ventilation volume is not entirely satisfactory since this does not take into consideration the fluctuations in oxygen absorption. We have therefore chosen to use as our criterion of changes in ventilation the ventilation equivalent for oxygen as defined by Knipping and Moncrieff ('32). This is the volume of air exhaled per 100 cc. of oxygen absorbed. These authors give 2.37 liters as the average figure for their normal subjects with a variation of approximately 25%. It will be seen from table 1 that the ventilation of our subjects as indicated by the ventilation equivalent was quite normal. There was a tendency in all subjects for the ventilation to increase slightly in the second half of the test. This tendency was slightly less marked in alcohol tests than in control tests.

We may sum up our results on basal tests with the statement that there was no indication of any specific dynamic action of alcohol during a period of 2 hours after its ingestion. There was no significant change in ventilation but there was a significant drop in the R.Q. which may be interpreted as an indication that alcohol is being oxidized. The validity of this last assumption is discussed later in this paper.

In table 2 we have recorded the oxygen consumption and ventilation equivalents during rest and work in all of the experiments of both series. It will be noticed by comparing the resting oxygen consumption of RCG, FAH and JRM, with the figures obtained in basal tests on these subjects, that sitting quietly on the bicycle caused a rise of from 5 to 8% in the oxygen consumption. Furthermore, the results obtained on these same subjects again fail to show any specific

dynamic action of alcohol. It may be, however, that more time is necessary for alcohol to produce its stimulating effect than was allowed in these tests. Two of the three subjects showed a slight drop in resting oxygen consumption after the ingestion of alcohol. Since both of these subjects reported subjective effects of sleepiness and general relaxation, it may

TABLE 2

The effect of dextrose and alcohol on the oxygen consumption and ventilation equivalent during rest and work

SUBJECT	TESTS		REST				WORK			
	Type	Number	Cubic centi-meter O ₂ per minute	SD.	Ventilation equivalent O ₂ ¹	SD.	Cubic centi-meter O ₂ per minute	SD.	Ventilation equivalent O ₂ ¹	SD.
RCG	C.	5	199.8	3.0	2.17	0.09	1031	59	2.10	0.04
	A.	5	196.2	4.9	2.20	0.10	950	36	2.05	0.04
	D.	3	206.7	4.1	2.33	0.18	1034	35	2.19	0.04
	D. + A.	3	209.1	1.9	2.08	0.17	951	36	2.11	0.01
FAH	C.	7	283.0	6.1	1.95	0.05	1335	46	1.99	0.04
	A.	7	286.5	5.7	1.79	0.01	1317	48	1.97	0.04
JRM	C.	3	252.0	0.7	2.46	0.01	1216	20	2.08	0.04
	A.	3	241.9	4.8	2.65	0.07	1206	56	2.10	0.01
CAV	C.	2	240.1	4.9	2.16	0.09	1143	56	2.10	0.02
	D.	2	266.8	7.6	2.03	0.13	1196	120	2.12	0.03
	D. + A.	3	255.4	5.4	2.01	0.11	1261	43	2.10	0.02
DUN	C.	2	246.9	3.8	2.47	0.11	1014	56	1.99	0.01
	D.	3	254.4	0.3	2.45	0.04	1087	36	2.03	0.04
	D. + A.	4	260.6	5.2	2.28	0.04	1133	41	1.97	0.03

¹ Liters of air exhaled per 100 cc. of oxygen absorbed.

be that this slight decrease in oxygen consumption is an indication of the narcotic effect of the alcohol. All three of the subjects that received dextrose showed a definite specific dynamic action following the ingestion of this substance. This amounted to about 4% in the case of RCG and DUN and about 8% with CAV. It is further significant that

the increase in oxygen consumption following the ingestion of dextrose plus alcohol was approximately the same in all three of these subjects as that obtained after the ingestion of dextrose alone. Thus it appears that alcohol had no specific dynamic action on any of our five subjects and we must conclude that if alcohol is capable of producing such an effect it must do so after an interval of more than 2 hours.

The oxygen consumption for work is characterized by an unusually high degree of variability. This is the result of a certain amount of unavoidable variation in the amount of work done during different tests. It is noticeable particularly in the case of RCG that the amount of oxygen used during work periods after alcohol and after dextrose plus alcohol, is nearly 10% less than the oxygen consumption during control tests. This is probably the result of a decreased amount of work performed in alcohol tests as a result of drowsiness brought on by the narcotic effect of the alcohol.

Ventilation equivalents show that there was no tendency toward over-ventilation in these tests since they are no higher for sitting at rest than they were in the basal tests. All of them except for the subjects JRM and DUN are lower than the average figures for normals given by Knipping and Moncrieff ('32). It is also worthy of comment that no rise in the ventilation equivalent occurred in the work periods. In fact in most cases there was a slight drop in the figure which would indicate that during the work periods oxygen was absorbed from the respired air more efficiently than at other times. Alcohol seems to have slightly decreased the ventilation equivalent in the cases of FAH, CAV and DUN. With subject RCG the administration of dextrose plus alcohol also produced the lowest ventilation equivalent ever observed on this subject. This effect is probably due to a depression of the respiratory center by the alcohol. The high degree of variability shown in the ventilation equivalents during rest, as indicated by the standard deviations given in the table, probably indicates that this center is easily affected by extraneous and fortuitous sensory impulses. The relatively

low degree of variability found in the ventilation equivalents during work would indicate that during periods of increased utilization of oxygen the respiratory center ceases to respond to such extraneous and fortuitous stimuli and is controlled almost entirely by the physiological needs of the organism.

Since in the latter part of this paper we shall be concerned largely with respiratory quotients and their interpretation it is important to know what, if any, changes in alveolar carbon dioxide occurred in the course of these experiments. No determinations of alveolar CO_2 were made in the first series

TABLE 3

The effect of dextrose and alcohol on the alveolar carbon dioxide during rest, work and recovery

SUBJECT	TESTS		CARBON DIOXIDE (PER CENT)					
	Type	Number	Rest	SD.	Work	SD.	Recovery	SD.
RCG	C.	1	5.43		5.23		5.29	
	D.	3	5.76	0.083	5.28	0.044	5.28	0.104
	D. + A.	3	5.71	0.118	5.54	0.063	5.30	0.121
CAV	C.	2	6.49	0.200	6.17	0.030	6.43	0.030
	D.	3	6.72	0.081	6.21	0.351	6.21	0.093
	D. + A.	3	6.66	0.067	6.41	0.123	6.33	0.030
DUN	C.	1	6.23		6.06		5.89	
	D.	3	6.51	0.193	6.16	0.090	6.14	0.079
	D. + A.	3	6.48	0.026	6.38	0.186	6.19	0.271

of experiments. However, during the second series of experiments the metal T to which the mouthpiece was attached was provided with a special valve which made it possible to collect alveolar air without the loss of any of the expired air except that which actually entered the collecting tube. The method used was essentially the same as that described by Carpenter and Lee ('33). By means of this device samples of alveolar air were taken at the end of the resting test, about 2 minutes after the end of the work test and again 12 minutes after the end of the work period. The results of these determinations are given in table 3. It will be noted that in all

cases there was a drop in alveolar CO_2 during the work period. In control tests there was usually a tendency for it to return to normal during the recovery period. When dextrose was given there was a slightly greater drop during work and when both dextrose and alcohol were given the drop was smaller and occurred more slowly than in the dextrose tests although it was still somewhat greater than that which occurred in control tests. The resting level of alveolar CO_2 was highest with all subjects after the ingestion of dextrose and lowest in control tests. The values obtained following the administration of dextrose plus alcohol were only slightly lower than those obtained with dextrose alone and the difference is probably not significant. However, in consideration of the reliability of respiratory quotients the most important factor is the change which occurs during the work period. If the drop which occurs following the ingestion of dextrose plus alcohol is significantly less than the corresponding change when dextrose is given alone, this would mean that less CO_2 was washed out of the blood in the dextrose plus alcohol than in the dextrose tests and therefore the lowering of the quotient after alcohol would have less significance. As has already been pointed out this is exactly what happens. However the differences are very slight. Rough computations show that the amount of CO_2 washed out during the dextrose tests in excess of the extra amount given off in alcohol tests is approximately 25 cc. This was given off over a period of more than 20 minutes which would mean that the excess amount retained in alcohol tests could not be more than $1\frac{1}{2}$ cc. per minute. Considering the relatively high level of oxygen consumption during the work period this amount of CO_2 would make a negligible difference in the respiratory quotient. We have calculated that this difference could not be greater than 0.002 and we therefore conclude that in so far as the alveolar CO_2 affects the quotient, differences greater than 0.005 between dextrose tests and dextrose plus alcohol tests are significant. Differences of the same magnitude between control and alcohol tests of the first series of experiments may also be assumed to be significant.

Table 4 shows the average values for the respiratory quotients obtained for rest and work and for the excess metabolism of work plus recovery. In the rest periods there was in all six experiments a significant lowering of the quotient following the administration of alcohol or of dextrose plus alcohol. In the subject RCG, who was used in both series of experiments, it is interesting to note that the lowering of

TABLE 4

The effect of dextrose and alcohol on the respiratory quotient of rest and work and on the excess respiratory quotient of work

SUBJECT	TESTS		REST	SD.	WORK	SD.	EXCESS TOTAL	SD.
	Type	Number						
RCG	C.	5	0.86	0.009	0.95	0.007	1.00	0.013
	A.	5	0.82	0.006	0.92	0.011	0.97	0.017
	D	3	0.87	0.009	0.98	0.004	1.04	0.006
	D. + A.	3	0.79	0.014	0.96	0.007	1.03	0.003
FAH	C.	7	0.80	0.008	0.96	0.015	1.04	0.019
	A.	7	0.76	0.005	0.93	0.012	1.02	0.014
JRM	C.	3	0.81	0.015	0.95	0.025	1.02	0.031
	A.	3	0.80	0.005	0.94	0.012	1.00	0.019
CAV	C.	2	0.84	0.025	0.94	0.001	0.99	0.010
	D	2	0.83	0.013	0.96	0.012	1.02	0.014
	D. + A.	3	0.79	0.012	0.93	0.004	0.99	0.004
DUN	C.	2	0.82	0.029	0.91	0.003	0.96	0.004
	D	3	0.86	0.002	0.95	0.009	1.01	0.018
	D. + A.	4	0.81	0.004	0.92	0.009	0.98	0.009

the quotient was considerably greater when dextrose and alcohol were given together than when alcohol alone was ingested. This is in agreement with the findings of Carpenter and Lee ('37). The administration of alcohol and dextrose plus alcohol was also followed without exception by a lowering of the quotient of both work and excess metabolism. These differences are of less magnitude than those for rest but we nevertheless believe them to be significant.

By assuming an average figure for protein metabolism (in the first series total urinary nitrogen was determined in all experiments and the protein metabolism calculated) we have calculated the quantity of alcohol oxidized in the various periods of all experiments. For these calculations Rosemann's formula as given by Higgins ('17) was used. The figures obtained in this way are given in table 5. The subject FAH was the only one for whom conclusive figures could be obtained for the utilization of alcohol during reclining tests.

TABLE 5

The utilization of ethyl alcohol during rest and work as calculated from changes in the respiratory quotient¹

SUBJECT	SUBSTANCE GIVEN	MILLIGRAMS OF ALCOHOL OXIDIZED PER MINUTE			TOTAL EXCESS ALCOHOL USED IN WORK AND RECOVERY IN MILLIGRAMS
		Reclining	Sitting	Working	
RCG	Alcohol ²	28.3	69.7	515.6
RCG	Dextrose + alcohol	No test	57.3	42.1	153.6
FAH	Alcohol	36.3	60.4	93.3	411.5
JRM	Alcohol ²	11.9	29.5	441.0
CAV	Dextrose + alcohol	No test	43.7	89.3	673.2
DUN	Dextrose + alcohol	No test	47.0	83.2	571.0

¹ The amount of alcohol oxidized per minute was calculated from the non-protein R.Q.'s and the non-protein oxygen consumption according to the following method: $\frac{\text{Control R.Q.} - \text{Alcohol R.Q.}}{\text{Control R.Q.} - 0.67} \times \text{cubic centimeters of O}_2 \text{ consumed per minute} = \text{the cubic centimeters of O}_2 \text{ used per minute in the oxidation of alcohol. This value divided by 1.4595 (the number of cubic centimeters of oxygen used in the oxidation of 1 mg. of alcohol), gives the number of milligrams of alcohol oxidized per minute.}$

² Data inconclusive.

This subject used about 66% more alcohol while sitting than while reclining. In five of the six experiments there was a marked increase in the utilization of alcohol in the working period over the amount used while sitting at rest. The averages for all six experiments are: sitting, 41.4 mg. per minute and working, 67.8 mg. per minute. This is an increase of a little more than 63%. The average total amount of alcohol used in the excess metabolism of work and recovery for all six experiments was 461 mg. The smallest value obtained was

153.6 mg. for the subject RCG when dextrose plus alcohol was given. This is less than a third of the amount of alcohol used by this subject when alcohol alone was ingested. This would seem to indicate that when there is an abundant amount of carbohydrate present the muscles use this in preference to alcohol. However, this conclusion is not borne out by the results obtained with CAV and DUN. These two subjects who received dextrose plus alcohol showed the greatest amount of alcohol used in the excess metabolism of work and recovery of any of the subjects. We are, therefore, not prepared at this time to make any conclusion as to what effect the simultaneous ingestion of dextrose and alcohol has on the utilization of alcohol during work. We do believe, however, that our experiments indicate that the energy of ethyl alcohol, whether given with dextrose or not, is available for use by the muscles in the performing of work.

The validity of the calculations by means of which the values given in table 5 have been derived rests upon two assumptions. First, that the observed differences between the quotients of control and alcohol tests are of sufficient magnitude to be statistically significant, and second, that these differences are the result of changes in the character of the fuel being oxidized by the various subjects, that is, the substitution of alcohol for carbohydrate and fat. In order to test the statistical significance of the results the probable errors of the differences between the respiratory quotients of the excess metabolism of work in control and alcohol tests have been calculated and the ratios between the observed differences and these probable errors determined. In the first series of experiments the observed difference was 2.1 times the probable error for the subject RCG, 1.25 times the P.E. for FAH and only 0.83 times the P.E. for JRM. In the second series of experiments the difference is twice the probable error for RCG, 3.0 times the probable error for CAV and 2.5 times the probable error for DUN. Now it must be remembered that a difference so slight as to be without significance in a single series of experiments becomes significant if it is shown that differences of this magnitude or

greater and in the same direction, invariably occur in similar series of experiments. Thus, if the changes of quotient were fortuitous in these experiments there would be one chance in two trials that the quotient would be lower in alcohol than in control tests, but that the quotients would be lower in the alcohol than in the control test for each of six different and distinct series of tests the chances are one-half raised to the sixth power or one-sixty-fourth. Thus slight differences such as those for JRM and FAH in the first series of experiments which would be without significance if considered separately become significant when considered in the light of the results obtained with the other subjects. Therefore these results justify the conclusion that there is a true lowering of the respiratory quotient of the excess metabolism of work following the ingestion of ethyl alcohol.

There are, however, many factors aside from the nature of the fuel being oxidized which will materially influence the value of the respiratory quotient. Among these may be mentioned the following: Changes in the ventilation rate, alterations in the acid base equilibrium, circulatory adjustments, and the interconversion of nutrients. It has already been shown by the ventilation equivalents given in table 2 that no changes in ventilation rate occurred with any of our subjects of such a magnitude as to produce variations in the quotients. Furthermore while the changes which occurred in the alveolar carbon dioxide in these experiments indicate that there was a shift in acid base equilibrium probably occasioned by an increase in blood lactic acid, the magnitude of this change was practically identical in control and alcohol tests and therefore without effect on the final results. Furthermore, while it is altogether possible that the quotients were affected by circulatory adjustments between the resting and work periods it seems entirely reasonable to assume that these adjustments were the same in the control as in the alcohol tests and that they also were without effect upon the final results. Finally, while it is true that the drop in the quotients observed may have been due to the interconversion

of the alcohol ingested to some other substance rather than to its direct oxidation, the substance produced from the alcohol would presumably be available for oxidation at some later time and the final results would therefore be the same as though it had been oxidized directly. For these reasons therefore, we feel justified in concluding that the drop in the respiratory quotient of the excess metabolism of work following the ingestion of ethyl alcohol is a valid, though presumptive, measure of the utilization of alcohol by the muscles in the performing of work.

SUMMARY

The effects of the ingestion of small amounts of ethyl alcohol alone and of dextrose plus ethyl alcohol on the respiratory metabolism of five male human subjects have been investigated by means of the Tissot-Haldane technic. The results justify the following conclusions:

1. Alcohol produced no specific dynamic action during the first 2 hours following its ingestion.
2. There was no significant change in the ventilation equivalent or in the alveolar carbon dioxide following the ingestion of alcohol.
3. Utilization of alcohol while resting was greater when given with dextrose than when given alone.
4. The rate of oxidation of alcohol in the body was higher in the sitting position than when reclining and still higher when working on the bicycle ergometer than when sitting at rest. The results are interpreted as presumptive evidence that the muscles are capable of using the energy of ethyl alcohol in the performing of work.

ACKNOWLEDGMENTS

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RECOVERY FOLLOWING SUPPRESSION OF GROWTH IN THE RAT ^{1, 2}

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ONE FIGURE

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The question of the influence of the length of the period of inanition upon the ability to resume growth after optimal nutrition has again been provided, is one which has long been discussed. The impossibility to completely suppress the growth impulse in rats, even by long-continued restriction of growth, has been stressed by Osborne and Mendel ('14, '15, '16), by Jackson ('25, '36) and by McCay, Crowell and Maynard ('35). On the other hand, Stewart ('16) and Jackson and Stewart ('20) found that there was a permanent stunting effect in rats underfed from weaning, the recovery upon refeeding being least complete in those animals whose growth was previously retarded for the longest time.

In the present study emphasis has been placed on the influence of the length of time of growth restriction prior to realimentation upon the subsequent development of the experimental animals. Two types of dietary procedures were used to retard the growth of young albino rats, namely, the restriction of a) inorganic salts and b) energy intake. Male rats of 100 gm. body weight (35 days old, approximately) were

¹Some of the data in this paper are taken from a dissertation presented by Miriam F. Clarke in partial fulfillment of the degree of doctor of philosophy, Yale University, 1933.

²Aided by a grant from the Russell H. Chittenden Fund for Research in Physiological Chemistry.

fed the low-salt diet for 3, 6 or 12 weeks during the period of restriction of growth. Controls of the same age were given either the adequate synthetic diet for similar experimental periods (age controls) or a complete synthetic diet limited in caloric intake to that of the low-salt rats whose food consumption was voluntarily reduced about 50% (calorie controls). It was thus possible to differentiate between the effects of the reduction of mineral salts and the limitation of the sources of energy. At the end of the period of the restriction of growth, both the low-salt rats and their calorie controls were realimented with an adequate synthetic diet fed ad libitum, the period of refeeding varying from 6 to 12 weeks as indicated by the tables and figure.

Besides weekly weights of the rats, the following values were recorded when each rat was killed: nose-anus length, weight of the kidneys, spleen, testes, femurs and incisors. Weights and other data concerning the bones and teeth have already been published (Clarke and Smith, '35; Clarke, Bassin and Smith, '36).

RESULTS

Weight and length

The retardation or cessation of growth was followed promptly by very rapid growth when the diet was made adequate (fig. 1). The curves show a striking difference between the recovery which followed a short period of suppression and that following longer periods. After 3 weeks stunting there was over-compensation both in the realimented low-salt rats and in the realimented calorie controls. Following restriction for 6 and 12 weeks, on the other hand, there was an apparently permanent stunting effect, especially noticeable in the low-salt rats. That the 'critical time' insofar as the completeness of recovery is concerned, lies between 3 and 6 weeks is shown by the curves c and d in figure 1. The rats represented by curve c, on the inadequate low-salt diet for 3 weeks, more than recovered weight lost through the restriction of mineral salts in the diet, indicated by the cross-

ing of the age control curve a. The same phenomenon of overcompensation is true of rats (curve b) restricted only in respect to the amount of energy consumed (calorie controls). The curve d represents the growth of animals in which the deleterious effects of 6 weeks on the mineral-restricted diet can be observed.

The experiments were not continued sufficiently long to determine whether the losses incurred by limiting the growth could ultimately be regained. During the last 4 weeks of the growth which followed the 6 weeks of restriction, the

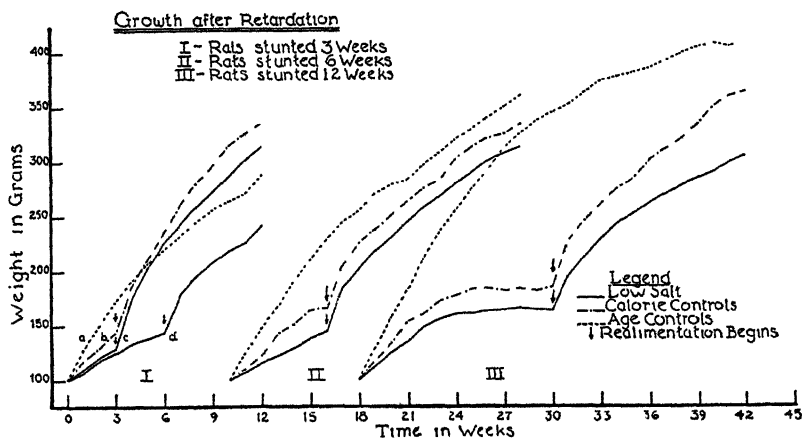


Figure 1

animals in all three groups (curve II, fig. 1) were growing at approximately the same rate, 1 gm. per day. At the end of the experiment the low-salt rats were 13% below the age controls in weight, while the calorie controls were only 7% below. Following the longer (12 weeks) period of limited growth, the initial acceleration upon realimentation was almost as striking and growth at a fairly rapid rate was sustained throughout the experiment (curve III, fig. 1). The rate of growth during the last 4 weeks (twentieth to twenty-fourth week) was approximately 0.5 gm. per day for the age controls, 1 gm. for the realimented low-salt rats, and 1.5 gm. for the realimented calorie controls. The possibility of

ultimately completely regaining body weight has therefore not been ruled out.

During the period of stationary weight the increase in length of the low-salt rats had practically ceased, while the calorie controls gained about half the amount gained by the age controls (table 1). The average length in millimeters

TABLE 1
Length of body (nose-anus)

WEEKS ON EXPERIMENT	NUMBER OF RATS IN GROUP		MEAN LENGTH	LENGTH COM- PARSED WITH AGE CONTROLS	WEIGHT LENGTH RATIO
Low-salt experiment					
3	Low-salt	10	<i>mm.</i> 176 ± 4.5^1	% 91	<i>gm./mm.</i> 0.73
	Age control	7	193 ± 3.3	100	0.92
	Calorie control	4	181 ± 4.3	94	0.80
6	Low-salt	11	183 ± 5.9	87	0.81
	Age control	7	210 ± 4.9	100	1.12
	Calorie control	4	199 ± 3.8	95	0.93
12	Low-salt	18	175 ± 8.0	76	0.89
	Age control	10	230 ± 8.6	100	1.30
	Calorie control	9	202 ± 4.8	88	0.91
	Weight control ²	10	180 ± 2.2	78	0.87
Realimentation experiment					
3 + 9 ³	Low-salt	10	227 ± 11.6	103	1.38
	Age control	8	221 ± 10.8	100	1.31
	Calorie control	4	236 ± 5.8	107	1.43
6 + 6	Low-salt	11	210 ± 8.2	95	1.15
	Age control	8	221 ± 10.8	100	1.31
6 + 12	Low-salt	10	225 ± 9.2	94	1.39
	Age control	7	240 ± 8.3	100	1.49
	Calorie control	4	233 ± 3.0	97	1.44
12 + 12	Low-salt	19	213 ± 8.6	86	1.44
	Age control	9	249 ± 4.9	100	1.65
	Calorie control	4	233 ± 8.9	94	1.56

¹ Standard deviation.

² Young rats, having same body weight as low-salt rats after 12 weeks.

³ Number following plus mark indicates number of weeks of realimentation.

Values in italics represent cases where the experimental mean is statistically different from the mean of the corresponding age controls.

of the low-salt rats at 12 weeks was actually less than that of the weight controls, animals of the same body weight but about 10 weeks younger. This apparent shrinkage may be due to a slight dorsal curvature characteristic of rats kept on the low-salt diet for extended periods. Arching of the back has been described for rats subjected to inanition by different methods (Aron, '14; Jackson, '15; Winters, Smith and Mendel, '27).

The response of the axial skeleton to adverse dietary conditions can be contrasted with that of the appendicular skeleton. In the former, growth ceased (table 1) while in the latter increase in length of the long bones proceeded at a rate about half the control rate both on the low-salt and the low-calorie regimes (Clarke, Bassin and Smith, '36). This difference was brought out by the study of Winters, Smith and Mendel ('27) with younger rats under similar experimental conditions in which it was observed that "while the animals made only 8.7 to 19.7 per cent of the normally expected gain in body length, the leg bones made 36 to 41 per cent." In the present experiment, with older rats, the growth in length was more completely inhibited while the increase in size of the long bones continued as with the young animals.

Comparison of the body length of experimental (low-salt and calorie control) rats with that of the age controls (table 1) shows that growth in length, considerably slowed down during the restricted period, accelerated rapidly during realimentation. In length as well as in weight the 3 + 9 group over-compensated. The high weight/length ratios for this group, however, indicate that recovery was less prompt than was that in body weight; this may arise from the fact that growth in length was affected relatively less than body weight during the period when development was checked. Ratios for rats stunted for longer periods before realimentation, show weight/length values lower than those of the age controls, indicating that the growth was distorted by a persistent increase in length when recovery of body weight was far from complete. Inorganic salts as the limiting factor had a

greater influence upon the subsequent growth in length than the mere reduction of the energy intake. This is understandable when one considers the pathology of the bones already described for rats under these experimental conditions (Arnim, Clarke, Anderson and Smith, '36), and the dependence of the length upon the growth of the skull and vertebral column. The response to realimentation of the axial skeleton, represented by body length, is similar to that of the long bones as representatives of the appendicular skeleton. Although affected unequally by faulty diets, they both responded by over-compensation after the shortest period of retardation, and by incomplete recovery after the longer periods.

The end of the experiment which was continued for the longest time found the realimented low-salt rats only 14% below the age controls in length whereas they were 25% below in weight. Moreover the linear growth was continuing at a fairly uniform rate, whereas the weekly weight increments were decreasing. Thus a persistent distortion of body proportions was brought about by the conditions of the experiment. That the phenomenon of growth in length may be less permanently impaired than growth in weight is more comprehensible when one considers the fact, pointed out by Outhouse and Mendel ('33), that the normal curve of linear growth tends to flatten at an earlier age than the curve for growth in body weight. At 100 gm., the starting weight of the present experiment, the normal male rats had attained about 70% of their final length, whereas they weighed at that time only 25% of their final weight.

A comparison of the weight/length ratios at the end of realimentation of the groups which had been restricted for 3 weeks with those restricted for 6 or for 12 weeks, emphasizes the fact that the distortion of development at the end of the experiment is definitely correlated with the length of time during which the growth was initially retarded.

Organ weights

The growth of the kidneys and the testes persisted during the salt-restricted period, resulting in organs which bear a distorted ratio to body weight (table 2). The spleen, on the other hand, atrophied during the period when salts were withheld (see also Swanson, Storvick and Smith, '36), a phenomenon which occurred also in the calorie-restricted rats, and appeared therefore to be associated with inanition. The kidneys tended to become hydrated (Swanson and Smith, '36) while the moisture of the spleen remained like that of the controls. The recovery during realimentation is indicated by increase of absolute weights of kidneys, testes and spleen, as well as by the return to nearly normal organ/body weight ratios. The average weights of these organs in the 3 + 9 groups (low-salt and calorie control) often exceeded by a small amount the corresponding value for the age controls. This is to be expected inasmuch as these organ weights bear a close relationship to body size (Donaldson, '24) and all are known to recover after inanition (Jackson, '25).

DISCUSSION

The rats in the present study may be divided into two categories, those which over-compensated after being restricted in growth and those which failed to overtake their unstunted controls. In each case the kidneys and testes as well as incisors (Clarke and Smith, '35), and femurs (Clarke, Bassin and Smith, '36) continued to grow to such an extent that they were large in proportion to body size at the end of the stunting period. The kidneys and testes as well as the spleen, returned promptly to normal size with respect to body weight when realimentation began. The difference between experimental (both low-salt and calorie control) rats and their age controls appeared to be, to a considerable extent, one of body fat, a difference that was soon made up when growth was allowed.

Whether the animals over-compensated in growth or failed to equal their controls in weight seems to be correlated with

TABLE 2
Weight of kidneys (g), testes (g) and spleen

WEEKS ON EXPERIMENT	GROUP	Low-salt experiment				Realimentation experiment			
		KIDNEY WEIGHT gm.	KIDNEY BODY WEIGHT × 100	KIDNEY MOISTURE %	TESTES WEIGHT gm.	TESTES BODY WEIGHT × 100	SPLEEN WEIGHT gm.	SPLEEN BODY WEIGHT × 100	SPLEEN MOISTURE %
3	Low-salt	1.256	1.09	75.9	2.040	1.6	0.301	0.23	78.0
	Age control	1.315	0.73	75.3	2.218	1.3	0.541	0.31	78.0
	Calorie control	1.080	0.75	75.6	1.941	1.4	0.389	0.27	77.9
6	Low-salt	1.309	0.88	76.4	2.210	1.5	0.288	0.19	77.9
	Age control	1.525	0.64	75.6	2.642	1.1	0.415	0.18	77.8
	Calorie control	1.196	0.65	76.2	2.373	1.3	0.407	0.22	77.5
12	Low-salt	1.295	0.85	76.9	2.249	1.3	0.313	0.21	77.3
	Age control	1.898	0.58	76.3	2.819	0.9	0.542	0.17	77.4
	Calorie control	1.214	0.66	75.8	2.469	1.3	0.312	0.17	77.2
	Weight control	1.145	0.73	76.4	1.885	1.2	0.487	0.31	78.0
Realimentation experiment									
3 + 9	Low-salt	1.704	0.56	76.2	2.879	0.9	0.536	0.17	77.5
	Age control	1.712	0.59	76.1	2.823	1.0	0.500	0.17	77.5
	Calorie control	1.873	0.56	75.7	2.679	0.8	0.512	0.15	77.3
6 + 6	Low-salt	1.519	0.64	76.4	2.598	1.1	0.443	0.18	77.6
	Age control	1.712	0.59	76.1	2.823	1.0	0.500	0.17	77.5
	Low-salt	1.758	0.56	77.0	3.062	1.0	0.431	0.14	77.2
6 + 12	Age control	2.026	0.57	76.4	3.172	0.9	0.473	0.13	77.2
	Calorie control	1.970	0.59	75.5	3.147	1.0	0.474	0.14	77.1
12 + 12	Low-salt	1.718	0.56	76.5	3.031	1.0	0.543	0.18	77.5
	Age control	2.224	0.54	76.7	3.300	0.8	0.792	0.19	77.2
	Calorie control	1.986	0.55	76.1	3.192	0.8	0.579	0.16	77.3

skeletal size. Although the skeleton is less readily influenced than other parts by nutritionally inadequate diets, and has often been known to persist in growth when the growth of the animal as a whole has ceased (Aron, '11, '14; Waters, '08), once it has become damaged, its subsequent growth and that of the body as a whole are seriously interfered with. Comparing the total growth with that of the skeleton as exemplified by the dimensions of the femur, fibula, radius and humerus (Clarke, Bassin and Smith, '36), it becomes apparent that the general growth follows closely the development of the skeleton. When the dimensions of these bones of the experimental rats exceeded those of the age controls in this study, the body weight also exceeded the control weight. This was true in spite of the fact that the bones of the low-salt rats actually weighed less than corresponding bones of age controls. Likewise experimental reduction in the size of the bones was associated with permanent dwarfing of the body as a whole. That the growth of the body depends on the skeletal pattern has been demonstrated by the experiments described by Jackson ('36) in which male rats failed to recover weight lost during 15 weeks of suppression of growth due to protein insufficiency. Here incomplete recovery in weight was associated with reduced skeletal size, exemplified by the nose-anus length; the weight of the skeleton plus musculature and of the humerus and femur. The females on the other hand, exhibited complete recovery with respect to both the total body growth and the skeletal growth.

The experiments described herein emphasize the fact that the sequel to suppressed growth insofar as recovery is concerned, depends on the length of time during which the growth was inhibited as well as upon the nature of the deficiency. Short periods of inhibition act as a stimulus for growth. This phenomenon of acceleration may be similar to that observed by Stearns and Moore ('31) in a child $3\frac{1}{2}$ years of age recovering from severe malnutrition due to multiple causes. Increase in weight of this child during the 9 months he was under observation was about nine times the normal increase for

that age, while growth in length exceeded the normal rate about fourfold. Thus they conclude that "if ever a child exerts his maximum capacity for growth, it is during rapid recovery from severe malnutrition."

The parallelism which exists between the skeletal pattern and the size of normal rats, growing at different rates, has been emphasized by Outhouse and Mendel ('33). Likewise, in experiments involving prolonged periods of stationary weight (McCay, Crowell and Maynard, '35), the "bone measurements fit the general picture of the final size attained. . . ." The early studies of Osborne and Mendel ('14, '15) contain no measurements of the skeleton, so that it would be impossible to determine from their data whether the complete recovery of body weight exhibited even after prolonged inanition was accompanied by complete return to normal skeletal magnitude. The tendency for the proportions of the body to remain in harmony despite adverse conditions is very great. Jackson and Stewart ('20) observed that the bodies of rats underfed from birth for long periods, then refed to maximum body weight, had nearly normal proportions, though they were permanently dwarfed. Whether the diet is lacking in the materials for skeletal growth, as in the low-salt diet, or only in total energy available, if it is fed sufficiently long the growth of the skeleton can be seriously interfered with. Both acceleration and retardation of skeletal development are reflected in somatic growth.

SUMMARY

The development of young rats following the suppression of growth due to restriction of mineral salts or energy intake was studied. The criteria used were increase in weight and length of the body and the weight of the kidneys, testes, spleen, femurs and incisors. The animals given the restricted diets for the shortest period, 3 weeks, over-compensated by exceeding their controls at the end of 9 weeks of refeeding. Rats stunted by either method for longer periods failed within the period of observation to regain losses suffered during

suppressed growth, although those restricted in energy intake alone made much greater gains in the time allotted and gave indications that complete recovery could ultimately occur. The weight of organs studied resumed a relationship to body weight like that of controls. Whether the stunted animals overtook and exceeded their controls in weight or failed to do so depended on the length of time their growth was suppressed which in turn influenced the growth capacity of the skeleton, both axial and appendicular.

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THE RELATIVE EFFECTS OF CERTAIN SACCHARIDES AND OF VITAMIN D ON MINERAL METABOLISM OF RATS ¹

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THREE FIGURES

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In a previous publication from this laboratory (Outhouse, Smith, Merritt and White, '37) the feeding of lactose was reported to have an accelerating effect on the calcification of bone which was not participated in by certain other naturally occurring carbohydrates. These results were in confirmation of those published earlier by Jarvis ('30), Kline, Keenan, Elvehjem and Hart ('32), and Whittier, Cary and Ellis ('35). In view of these findings, it was considered that studies involving the metabolism of the bone-building elements might aid in explaining the manner in which this calcifying action is brought about. The present paper deals with studies designed to show the influence of this carbohydrate on calcium, phosphorous, and magnesium metabolism. The effects were compared with those obtained by the use of two other commonly used carbohydrates (starch and sucrose) and the calcifying agent par excellence, vitamin D. In general, the data indicate that both lactose and vitamin D acted similarly in that they stimulated the retention of all three elements in excess of that induced by starch or sucrose, but differed primarily in that more of calcium and of phosphorous was stored under the influence of vitamin D.

¹ A portion of these data was presented before the American Chemical Society, Cleveland, 1934.

EXPERIMENTAL

The procedures used in this study were those described for series 2 in the aforementioned paper from this laboratory. In fact, the bone-ash values of the twelve groups of rats herein reported are presented in table 4 of that paper (groups 4203 to 4738 inclusive). It should be reiterated that control-feeding technic was adapted to groups of four litter-mate rats, each of the four animals receiving a different ration but an equivalent number of calories and practically the same quantity of mineral salts. The rats were maintained on the rations for 28 days and were 56 days of age when the experiment was terminated.

Between the twelfth and twenty-fourth days of the experiment the excreta were collected for analysis. Ferric oxide was fed as a feces marker. During this 12-day period the rats were placed in cylindrical Pyrex glass cages of the type described by Smith and Brooke ('30-'31). Both urine and feces fell upon filter paper (Whatman no. 40) which had been treated according to the method of Brooke and Smith ('33) with 2 N. acetic acid and thymol. The feces were collected daily and at 4-day intervals the cages were carefully washed with redistilled water. The combined washings, together with the filter paper, were digested on a steam bath, filtered, and washed with redistilled water until there was no trace of residual urine. This filtrate was then evaporated to a convenient volume. Urine and feces were digested separately in dilute acid according to the method of Stearns ('28-'29). Both digests were made to volume, and aliquots were used for analysis.

Calcium and magnesium were determined on the excreta by the procedure of Morris, Nelson and Palmer ('31) and on the rations by the gravimetric method of McCrudden ('11-'12). In the determination of phosphorus the method of Meigs, Blatherwick and Cary ('19) was used for the rations and that of Fiske and Subbarow ('25) for the excreta.

RESULTS

Bone-ash values. The bone-ash values are given at the top of the columns in figures 1 to 3. In each of the twelve groups, the cod liver oil ration and the lactose ration induced the deposition of more ash than did the starch or the sucrose diets of the litter mates. A comparison of the lactose and cod liver oil rations showed that in seven of the twelve groups the animal on the latter ration had more ash in its bones than had its litter mate. For this comparison (Student, '15-'17) the statistical odds of 27 to 1 were too low to be more than suggestive that cod liver oil, at the levels fed, was the better calcifying agent.

Weight of feces. The fecal material appeared to be bulkier in the case of the lactose-fed rats. Upon removal of the moisture in vacuo, the dry weight of the stools was found to be greatest for the rats receiving lactose in each of the ten litter-mate groups and least for those receiving cod liver oil in nine of the ten groups. Statistically, these differences were significant. No appreciable disparity could be found between the weight of feces of litter mates on the other two rations. The greater weight of the stools of the lactose-fed animals could not be explained on the basis of excessive loss of calcium or phosphorus whereas the low values found for the cod liver oil animals were paralleled by a decreased excretion of these two elements. A study was not made of the bacterial content of the stools as was done by Mitchell ('26-'27 a, b).

CALCIUM METABOLISM

Calcium intake. The data for the calcium metabolism of the individual animals are presented graphically in figure 1. The calcium content of the rations approximated 0.50%. Considerable variation occurred in the amount ingested by the twelve different groups. For the 12-day period the intake ranged from 261 to 500 mg.

Calcium retention. For the different rations, characteristic variations in the retention of calcium were found. The rats fed either lactose or cod liver oil retained more calcium than

did their litter mates on either the sucrose or the starch rations in each of the twelve groups. On comparing these two calcifying agents, however, the latter was found to favor greater retentions in all but three of the groups. Statistical odds of 293 to 1 indicate a high degree of probability that the addition of vitamin D stimulated the greater storage of calcium. No difference could be found between the retention by litter mates on the starch and sucrose rations. For the rats on a given ration the retention of this element was definitely related to intake as indicated by high coefficients of correlation² between these two factors.

The amount of calcium retained in relation to that ingested was fairly consistent for the rats on a given ration. The average values for the starch, the cod liver oil, the lactose, and sucrose rations were $37 \pm \text{P.E. } 4.5$, $61 \pm \text{P.E. } 3.0$, $51 \pm \text{P.E. } 5.6$, and $38 \pm \text{P.E. } 4.2\%$ of the intake respectively. Statistically significant differences existed between these values for all the rations except between the values for the starch and sucrose rations. These data indicate that, in stimulating calcium storage, the lactose ration was 38%, and the cod liver oil ration 62% more effective than the other two rations.

Urinary calcium. The quantity of calcium excreted by way of the kidney was very slight and bore no relationship to the total amount eaten or excreted. However, it was influenced by the type of ration eaten. The ingestion of lactose and of cod liver oil was accompanied by a greater loss of calcium in the urine than was the ingestion of sucrose. A similar relationship occurred in a comparison between the values of the lactose and starch rations but not between those for the cod liver oil and starch rations.

Fecal calcium. The differences between the effects of the four rations on the amount of calcium lost in the feces showed the same relationships as was found in their effects on total calcium retention. Thus, on both the lactose and the cod liver oil rations, litter mates excreted less fecal calcium than those

² Correlation coefficients were considered to be significant if the values were four times as great as the probable error.

on the other two rations in 100% of the cases. In comparing the two calcifying agents the statistical odds were 521 to 1 that less calcium was excreted by the animals receiving cod liver oil. For any one ration the fecal calcium values tended to be related to the quantity of calcium eaten, but the correlation coefficients were significant only for the starch ration and for the cod liver oil ration.

PHOSPHORUS METABOLISM

Phosphorus intake. The data for phosphorus metabolism are given in figure 2. The phosphorus content of the ration approximated 0.63% and the quantity consumed during the 12-day period varied, between the twelve groups, from 196 to 580 mg.

Phosphorus retention. Cod liver oil ingestion was associated consistently with a greater retention of phosphorus than was found on the other three rations. (In one litter-mate group the lactose-fed rat stored more.) On the other hand, the lactose-fed rats had a tendency to store more of this element than did their litter mates on the starch and sucrose rations. Statistical odds of 36 to 1 and 525 to 1, respectively for these last two comparisons were high enough only in the latter case to give practical certainty that a true difference existed in the amount of phosphorus stored.

The retentions of this element by the animals on a given ration tended to be dependent on the quantity of phosphorus consumed. High correlation coefficients between these variables were obtained for all the rations. The retentions, in terms of per cent of intake, gave average values of $17 \pm \text{P.E. } 4.5$, $27 \pm \text{P.E. } 3.5$, $22 \pm \text{P.E. } 4.0$, and $18 \pm \text{P.E. } 3.5$ for the starch, the cod liver oil, the lactose, and the sucrose rations respectively. Significant differences between these values were found only for the cod liver oil ration in relation to the other three diets.

Urinary phosphorus. For urinary phosphorus the only significant difference between the animals of a quartet was found in the case of the cod liver oil-fed rats. These showed greater

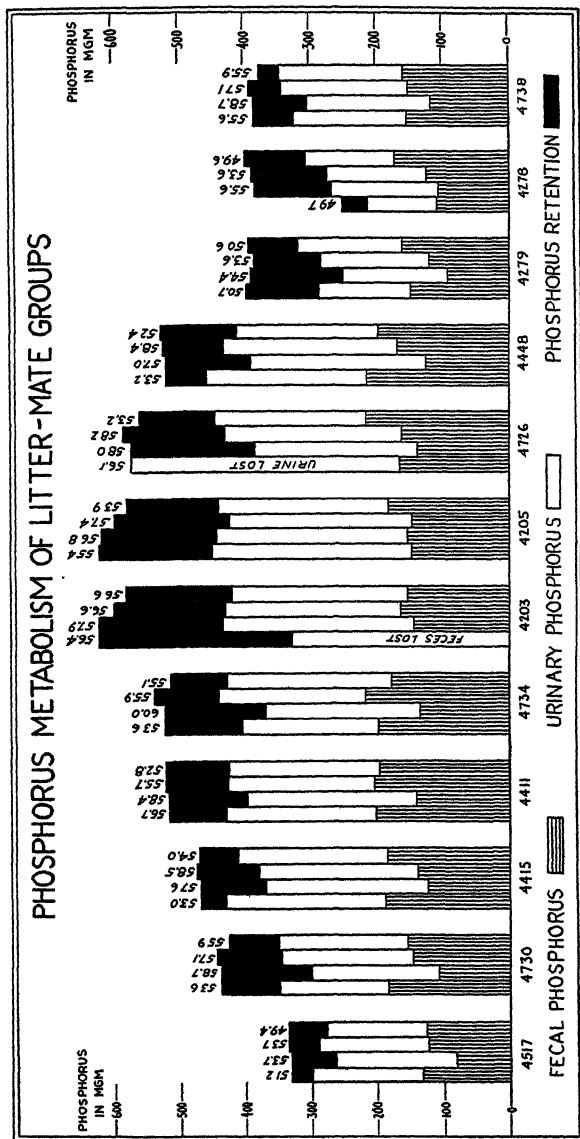


Fig. 2 The slight inequality in the amounts of the phosphorus consumed by the litter mates (as noted particularly for groups 4203 and 4205) was due to small differences in the mineral content of the rations in the quantity of food eaten.

In group 4278 the rat on the starch ration developed anorexia during the latter part of the metabolism period. Therefore the food of the other three animals was equalized with that of their litter mates in group 4279.

excretions than did the litter mates fed either the starch or sucrose rations (odds of 132 to 1 and 908 to 1 respectively) but no greater than did those fed the lactose diet.

In contrast to urinary calcium, the amount of urinary phosphorus, was related to the quantity of phosphorus ingested as indicated by high correlation coefficients.

Fecal phosphorus. The ingestion of lactose tended to reduce the excretion of phosphorus in the stools below that obtained on the starch and sucrose rations, but the odds were very low (36 to 1, and 49 to 1, respectively). The rats on the cod liver oil ration, however, excreted much less phosphorus than their litter mates on the other rations with only two exceptions (the odds were 4999 to 1, or greater). The quantity of phosphorus in the stools varied with phosphorus intake as shown by very high correlation coefficients.

MAGNESIUM METABOLISM

Magnesium retentions. The magnesium content of the rations approximated 0.083%, and the quantities consumed by the twelve litter-mate groups during the 12-day period ranged between 42 and 73 mg. Of the forty-seven animals (for which complete metabolic data were available) twelve lost more of this element than they ingested (fig. 3). These negative balances were not confined to the animals on any one ration or to any one litter (in only one quartet were all members in negative balance). Neither were they related to the quantity of food eaten, inasmuch as a correlation could not be found between the level of intake of magnesium and the amount stored in the body. With respect to the retentions of this element a statistical difference did not exist between the pairs consuming the two rations which had been shown to possess calcifying properties (odds 15 to 1). Both induced greater storage than did the starch ration but in comparison with the sucrose ration, the lactose, and not the cod liver oil ration, caused a slight, though significant, increase in storage.

Urinary magnesium. The lactose ration stimulated the excretion of a significantly greater quantity of magnesium

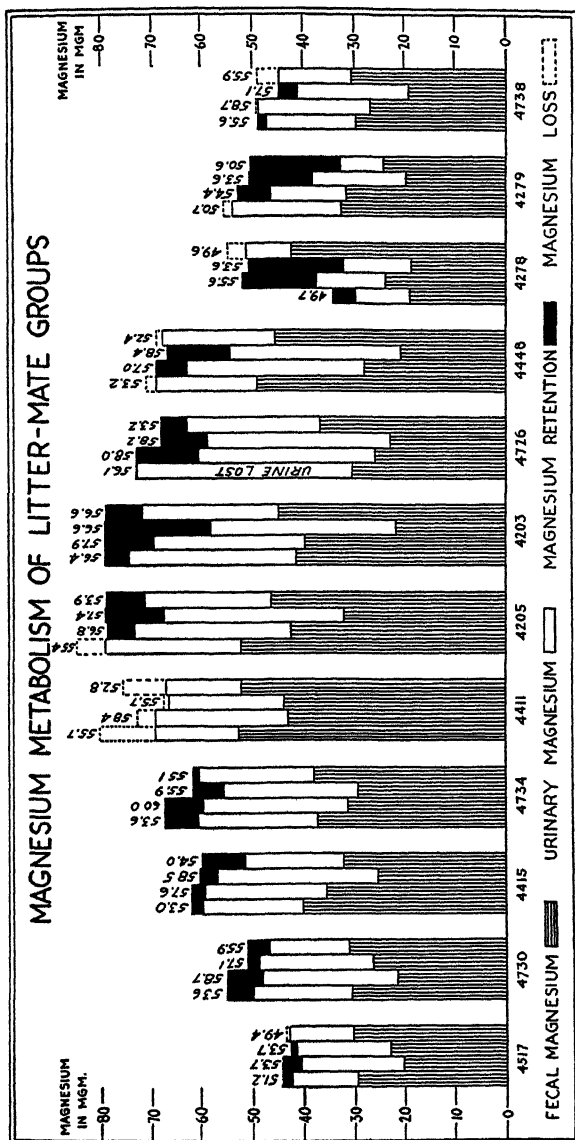


Fig. 3 The slight inequality in the amounts of magnesium consumed by the litter mates (as noted particularly for groups 4203 and 4205) was due to small differences in the mineral content of the rations and not to a disparity in the quantity of food eaten.

In group 4278 the rat on the starch ration developed anorexia during the latter part of the metabolism period. Therefore the food of the other three animals was equalized with that of their litter mates in group 4279.

in the urine than did the starch or sucrose ration. The ration containing vitamin D differed from the one containing lactose in that only in comparison with the sucrose ration did a greater loss of magnesium in the urine occur. These were the only significant differences found for any of the possible comparisons. For each of the four groups, the quantity lost through this channel showed a close correlation with the amount ingested.

Fecal magnesium. The animals on the two calcifying rations excreted less magnesium by way of the gut, than did their litter mates on the other two rations. For the first two rations, the fecal magnesium was less in the case of the rats receiving lactose. The quantity excreted showed a significant correlation with intake only in the case of the animals on the starch ration.

DISCUSSION

This metabolism study has shown that the ingestion of lactose caused a greater quantity of calcium, phosphorus, and magnesium to be stored in the body than was found in litter mates on the starch and sucrose rations. These more favorable retentions were, in all cases, the result of smaller excretions by way of the gut. Cod liver oil produced these same effects with the exception of the magnesium retentions of the cod liver oil-fed animals which were no greater than those of the sucrose-fed animals. The only consistent difference that could be found between these two calcifying agents was that the fecal excretion of all elements was less, and the total retentions were greater in the animals receiving vitamin D.

An analysis of the data was undertaken to determine the extent to which the retention of these elements was concerned with the calcification of the bones. No correlation was found between bone-ash values and the retention of calcium, phosphorus or magnesium when the data for the animals on any one ration were considered. However, for a litter-mate group, within which variations due to food intake and litter

differences were eliminated, a definite relationship existed for calcium and for phosphorus. Of the fifty-six possible comparisons³ (i.e., starch vs. cod liver, starch vs. lactose, lactose vs. cod liver, lactose vs. sucrose, and sucrose vs. cod liver oil) there were fifty-three that showed not only higher bone-ash values but also a greater retention of calcium. Greater phosphorus retentions were also associated with the higher bone-ash values induced by cod liver oil in twenty-eight of the twenty-nine instances. On the other hand, in only fifteen of the twenty-two instances (69%) in which the ingestion of lactose was attended by a higher ash content of the bone was there a parallelism in phosphorus retentions. In respect to magnesium, the relationship held for cod liver oil in only twenty-one out of thirty instances and for lactose in only seventeen of twenty-six instances (i.e., 70 and 65% respectively). From these data it would appear that the calcification, which was induced by vitamin D, was the result of a stimulation to the storage of both calcium and phosphorus, whereas that attending the ingestion of lactose was due primarily to calcium. The inconsistent parallelism between magnesium retentions and bone-ash values indicates the possibility that a part, at least, of the excess magnesium retained by the animals receiving either of the calcifying agents over that retained by the animals on the other rations may have been stored in some tissue other than bone.

SUMMARY

1. The influence of starch, lactose, sucrose, or cod liver oil rations on calcium, phosphorus and magnesium metabolism was studied.
2. Control-feeding technic was adapted to groups of four litter-mate rats, each of which received one of the above rations.
3. The lactose and cod liver oil rations caused greater retentions of all three elements than did the starch or sucrose rations with one exception, i.e., the cod liver oil ration caused no greater magnesium storage than did the sucrose ration.

³ There were fewer than the sixty possible comparisons due to the loss of part of the metabolic material.

4. Greater increments in retentions were obtained in the cod liver oil-fed animals than in the lactose-fed rats.

5. The higher bone-ash values resulting from the feeding of vitamin D were accompanied by greater retentions of calcium and phosphorus. Similar results were found for the lactose animals except in the case of phosphorus, for which only 69% of the instances showed a parallelism to bone ash.

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THE USE OF FIBRIN IN SYNTHETIC DIETS

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ONE FIGURE

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Since the so-called synthetic or purified diets have been employed for experimental studies casein has been the most commonly used protein. Several factors have led to this choice, viz., a) the facility with which casein can be obtained in a relatively pure state, b) its comparatively low cost, c) the readiness with which it can be extracted with various solvents for the purpose of purification, d) the ease with which it can be ground and incorporated into the ration, e) its high biological value and finally f) its freedom from any toxic principle even when fed at high levels. There are times, however, when casein is not entirely satisfactory and it is desirable to use some other protein. For example, casein is a conjugated protein containing phosphoric acid and consequently cannot be used in low phosphorus diets. Also casein as usually obtained in the crude state contains many impurities of nutritional significance which would be expected as its original source is milk. Although, as previously stated, casein can be easily extracted with a wide variety of solvents, it is not a simple problem to remove all of these impurities. Day and Darby ('37) have recently emphasized the difficulties of purifying casein sufficiently for use in the production of cataracts in rats resulting from a deficiency of riboflavin. They have pointed out that some samples of commercially purified 'vitamin-free' casein are unsatisfactory for this purpose. In

this and similar cases it would be well to have another protein to use instead of casein or at least to compare with casein when the latter is suspected of giving undesirable results. There are probably numerous other instances in which the use of some protein other than casein would be advantageous providing it also had most of the desirable properties of casein listed above.

Our interest in this problem arose during an attempt to prepare a diet very low in phosphorus. After considering the various proteins available, blood fibrin was chosen as being most likely to fulfill the larger part of the desired qualifications. Beef-blood fibrin is now available in any quantity and at a price comparable with that of casein, and the following experiments show that it can be used readily as the source of protein in various synthetic rations.

EXPERIMENTAL

Beef-blood fibrin is easily ground to any desired degree of fineness and can be extracted with alcohol or ether without difficulty. With water it swells more than casein and tends to form a jelly. In the crude, air-dried state it contains approximately 13.3% nitrogen, 5.0% alcohol-ether soluble extract, 0.08% calcium and 0.10% phosphorus. After extracting with alcohol and ether the nitrogen is increased to 14.3%, the phosphorus reduced to 0.085% and the calcium remains unchanged. Very little material is extracted by ether following hot-alcohol extraction in a Soxhlet apparatus.

As a preliminary growth experiment the crude product was fed at a level of 18% with a basal diet of dextrinized starch 70, yeast 6, salt no. 40 (Steenbock and Nelson, '23) 4, agar 2 and cod liver oil.¹ On this diet young rats grew at a rapid rate and appeared normal. In subsequent experiments the growth-promoting properties of alcohol-extracted fibrin were compared at different levels with alcohol-extracted casein. The results are given in figure 1. As can be seen the biological

¹ Whenever cod liver oil was included in the diet 0.25 cc. was given directly three times per week.

value of fibrin, as determined by this method is equal to, if not somewhat greater than, casein. When these two proteins were fed at equal-weight levels the fibrin diets contained slightly more nitrogen than the casein diets as the former protein had a little higher content of nitrogen than did the latter.

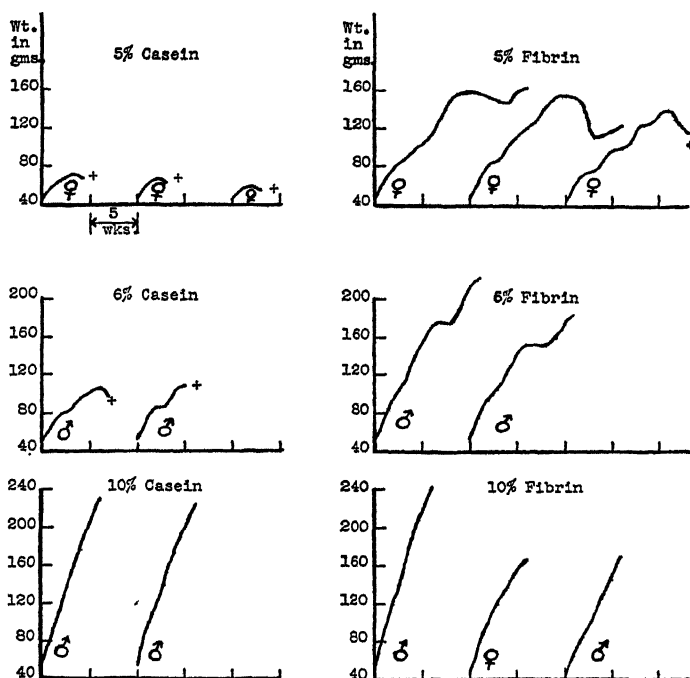


Fig.1 The comparative growth of rats produced by various levels of alcohol-extracted casein and alcohol-extracted fibrin fed in addition to a basal diet of dried yeast 6, salt no. 40 (Steenbock and Nelson, '23) 4, agar 2, dextrin to make 100 and 0.25 cc. of cod liver oil three times weekly. + indicates death of animal.

Having determined that fibrin could support good growth at comparatively low levels the next step was to ascertain if it produced any toxic symptoms when given in large amounts. For this purpose a diet similar to that used by Parsons ('31) in her studies on the toxicity of egg white was employed. It was composed of the following ingredients expressed in per cent, fibrin (alcohol-extracted) 66, yeast 20, wheat embryo 10,

salt no. 40 (Steenbock and Nelson, '23) 4 and cod liver oil. Four rats (two males and two females) were fed this diet for 12 weeks. At the end of this time the animals appeared perfectly normal. They had increased their weight from an average of 55 gm. to 237 gm. during the experimental period. If the results of Parsons are due to the presence of a toxic factor in egg white it is apparent that no similar substance is present in fibrin in quantities sufficient to interfere with its use in feeding experiments.

Studies were also made upon the use of fibrin in diets intended for vitamin investigations. The crude unpurified material contains sufficient vitamin A to produce nearly normal growth when the protein is fed at a level of 18% and no other source of vitamin A is added. Extraction with alcohol, either by cold percolation or in a Soxhlet apparatus, appeared to completely remove this vitamin. Rats given a ration containing 18% alcohol-extracted fibrin with no added vitamin A developed typical ophthalmia within a period of about 5 weeks which was followed in a short time by death.

In a similar manner it was found that the alcohol-extracted fibrin contained little or no vitamin B₁. When 1% of liver extract² furnished the other vitamin B factors and 18% of alcohol-extracted fibrin was the source of protein, rats failed very rapidly and developed symptoms typical of B₁ deficiency, including the characteristic convulsions, within 5 or 6 weeks. For purposes of comparison diets containing purified casein instead of fibrin were fed simultaneously with the fibrin-containing diets used in the vitamin A and B₁ deficiency studies. No differences in the results obtained with the two proteins were detectable.

As fibrin contains considerably less phosphorus than casein it was of interest to study the possibility of using fibrin in rachitogenic diets. Various modifications of the widely used Steenbock and Black ('25) rachitogenic diet no. 2965 were employed in these experiments. In one series of experiments conducted simultaneously three litters of rats at about 25 days

²Eli Lilly and Co., no. 343.

of age were divided into four groups and given diets as follows:

Group I. Steenbock-Black diet no. 2965.

Group II. Yellow corn 86, crude fibrin 10, CaCO_3 3, NaCl 1.

Group III. Same as group II except alcohol-extracted fibrin replaced the crude fibrin.

Group IV. Commercial yellow cornmeal 76, wheat embryo 10, alcohol-extracted fibrin 10, CaCO_3 3 and NaCl 1. This last diet is based on a rachitogenic diet previously used in this laboratory (Jones, '34). Within a short time all of the animals with the exception of those of group II began to show definite enlargement of the wrists and at the end of 3 weeks, when all of the animals were bled to death, the enlargements were very pronounced. At the termination of the experiment the blood of each group was pooled and the sera analyzed for calcium by the method of Clark and Collip ('25) and for phosphorus on the calcium-free filtrate by the method of Gunther and Greenberg ('29). The right femur of each animal was removed and the amount of ash in the lipid-free bone was determined and a wrist bone from each animal was examined by the 'line-test' technic. This experiment was repeated as above with two more litters of rats but with a different sample of fibrin. The chemical analyses from both experiments, which are summarized in table 1, and the results from the examination of the wrist bones confirmed the clinical findings. That is, all of the animals except those receiving the unextracted fibrin (group II) showed a marked degree of rickets.

From these experiments it is clear that fibrin can be used as a protein in rachitogenic diets but it first must be extracted with alcohol or similar solvent. Judging from the comparatively small reduction in the amount of phosphorus caused by the extraction it is assumed that the anti-rachitic properties of crude fibrin are due to the presence of vitamin D and not to its higher content of phosphorus. Furthermore, we have frequently observed that if liberal amounts of vitamin D are given in conjunction with a high-calcium rachitogenic diet, the calcium of the blood serum may be considerably above normal.

From table 1 it can be seen that in both experiments the calcium of the serum of the animals receiving the crude fibrin was above 15 mg. per 100 cc.

The fibrin-containing diets used in these experiments contained slightly less phosphorus than the Steenbock-Black diet as the phosphorus content of fibrin is less than that of wheat gluten, and the former protein comprised only 10% of the ration instead of the 20% level at which the wheat gluten is fed. Due to the lower content of phosphorus the fibrin-containing diets should have somewhat greater rachitogenic properties than the Steenbock-Black diet. There is some evidence

TABLE 1

The comparative influence of the various fibrin-containing diets and the Steenbock-Black diet on bone ash and the calcium and phosphorus concentrations of the serum

GROUP	NUMBER OF ANIMALS	GAIN IN WEIGHT ¹	BONE ASH		SERUM	
			Weight ¹	Per cent ¹	Ca	P
		<i>gm.</i>	<i>mg.</i>		<i>mg. per 100 cc.</i>	<i>mg. per 100 cc.</i>
I-A	4	17	17.8	25.5	12.1	2.8
I-B	3	22	24.3	28.4	12.2	2.1
II-A	4	28	40.9	40.6	15.2	6.3
II-B	3	31	48.4	42.9	15.8	5.7
III-A	4	20	16.0	20.8	12.4	1.6
III-B	3	20	21.1	23.8	12.5	2.4
IV-A	5	13	15.7	23.1	12.1	1.9
IV-B	3	19	20.5	24.0	11.5	2.4

¹ Average of all animals of each group.

to indicate that this is true. A comparison of the amount and percentage of ash of the femora of the animals of groups I and III (table 1) show that within each experiment there was more calcification on the Steenbock-Black diet than on the diet containing fibrin. When the fibrin makes up 10% of the ration the growth is comparable to that on the Steenbock-Black diet as is shown by table 1. Additional results on the use of fibrin in rachitogenic diets will be reported in the future.

All the experiments reported above, except the one in which the fibrin was fed at a high level to study its possible toxicity,

were repeated using two different shipments of fibrin. There were no significant differences in the results obtained with the two different samples. Except for the few experiments on B₁ these investigations have not included a study of the possible use of fibrin in work concerning the vitamin B complex. It is highly probable, however, that it may prove useful as casein contains rather large amounts of these factors which are not easily removed.

SUMMARY

Crude beef-blood fibrin as the only source of protein in a synthetic diet supports growth at a level comparable to that of casein. Fibrin can be extracted with organic solvents as readily as casein. With water it swells more than casein. It can be easily dried, ground and incorporated into synthetic diets. Before purification beef-fibrin contains liberal quantities of vitamins A and D which can be removed by extraction with alcohol. Alcohol-extracted fibrin is practically free of vitamin B₁. Fibrin is low in phosphorus and consequently can be used in rachitogenic diets. As fibrin is available at a price approximately the same as casein it may be used in synthetic diets when casein is not suitable.

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LACK OF VITAMIN C IN THE DIET AND ITS EFFECT ON THE JAW BONES OF GUINEA PIGS¹

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TWO FIGURES

(Received for publication September 25, 1937)

INTRODUCTION

While working with the teeth of guinea pigs which had been kept on the Sherman, LaMer and Campbell ('22) vitamin C free diet and others kept on the same diet with different vitamin C supplements it was noticed that some of the jaws had dark circular areas on the exterior surface of the mandible at the bases of the cheek teeth, and others were without these areas.

PURPOSE

The purpose of this experiment was to discover the nature of these areas and find if possible a correlation between them and the diet used.

REVIEW OF LITERATURE

Dental caries, alveolar resorption and irregularities of the teeth themselves were produced in guinea pigs fed on a diet without vitamin C (Howe, '20, '21; Höjer, '26; Key and Elphick, '31; and others).

Decalcification of the teeth and some parts of the bones has been reported in guinea pigs as a result of scorbutic feeding (Howe, '22; Zilva and Wells, '19; Höjer and Westin, '25; Key and Elphick, '31; and Dann and Cowgill, '35).

¹Contribution no. 161, department of zoology and no. 75, department of home economics.

On the other hand it has been claimed that "no apparent morphological change takes place in the hard tissues already formed" (Fish and Harris, '34).

The formation of the teeth in the guinea pig and the formation of bone in which the teeth are located take place at about the same time (Harman and Smith, '36).

MATERIALS AND METHODS

All animals used in this experiment were classified into three groups which were fed as follows: 1) Vitamin C free diet (Kramer, Harman and Brill, '33)—negative controls. 2) Vitamin C free diet plus 3 ml. fresh orange juice daily per 300 gm. body weight. 3) (a) Vitamin C free diet plus greens ad libitum. (b) Ibsen diet (Harman and Prickett, '32)—positive controls.

The Ibsen diet is composed of water, alfalfa hay, greens and a rolled oats mixture. The greens consist of fresh alfalfa in summer and sprouted oats in winter. The rolled oats mixture is as follows:

	<i>Pounds</i>
Rolled oats	50
Wheat shorts	1½
Skimmed milk powder	1½
Tankage	¼
Bone meal	¼
Table salt	¼
100 D brewer's yeast	¼

Some animals died and others were killed at stated times. The jaws were preserved in 95% alcohol.

Observations

The dark areas at the bases of the cheek teeth were irregularly circular in shape and varied from 2 mm. to 2½ mm. in diameter (fig. 1). They were so soft that they could be pierced easily with a needle. The bone had the appearance of a sponge. In extreme instances the entire area appeared as a hole.

Usually the jaws having the dark areas were crumbly in the external alveolar part of the mandible in the region of the cheek teeth. More often this condition was present near the posterior cheek teeth but in some instances the entire external alveolar area was so brittle and there was so little hard substance that there was scarcely enough bone remaining to form sockets for the teeth (fig. 2).

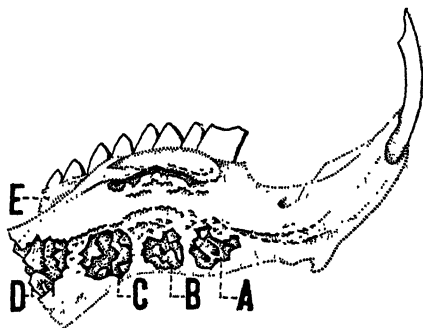


Fig. 1 Drawing of left lower jaw of T-18, from caudad lateral surface showing the soft areas at the base of the cheek teeth. A, B, C and D the soft areas at the base of the first, second, third and fourth cheek teeth, respectively. E, alveolar bone.



Fig. 2 Drawing of left lower jaw of T-18 from cephalad lateral surface, showing the condition of the alveolar bone. F, crumbly alveolar area. G, line of the gum.

Negative controls

Fifteen guinea pigs were used as negative controls. The ages of these animals when put on the diet ranged from 20 days to 3 years and 3 months. The length of time on the diet varied between 26 and 52 days. Five of the animals died between 26 and 40 days on the diet. One died at 41 days and another died at 42 days. One was in a dying condition at 41 days when it was killed. Another had been too weak

to stand for 10 days when it was killed after being on the diet for 52 days. Six of the animals were killed at 28 days.

All the animals had either the soft area at the base of one or more cheek teeth or crumbly alveolar bone or both, whether old or young when they were put on the diet, even if they had been on the diet only 28 days.

All the younger animals, those which were 4 months of age or less when put on the diet, had a soft area at the base of each cheek tooth as well as crumbly alveolar bone. The alveolar area in the region of the third and fourth cheek teeth was affected the most. Both of the very old animals had soft crumbly alveolar bone and a soft area at the base of the third and fourth cheek tooth.

Animals which received the basic diet and 3 ml. of orange juice per 300 gm. of body weight

Twenty-two animals received the basic diet plus 3 ml. of orange juice per 300 gm. of body weight. Their ages when put on the diet varied from 21 days to 10 months. None of the animals of this group died while on the experiment. Two of them were pregnant when killed and two gave birth to young a short time before they were killed. With two exceptions the alveolar and basilar portions of the jaws were firm. One animal had a small soft area at the base of the third cheek tooth and another had a small soft area at the base of the fourth cheek tooth. All other parts of the jaws of these animals were firm and strong. Both of these animals were old, 2½ and 3 years, respectively.

Positive controls (a) vitamin C free diet plus greens ad libitum

Of the five animals receiving the vitamin C free diet plus greens ad libitum all were put on the experiment at birth. The length of time on the diet ranged from 14 to 75 days. None of the animals died. One was pregnant when killed. One had a slightly crumbly alveolar bone. All the others had all parts of the mandible firm and strong.

Positive controls (b) Ibsen diet

Of the animals on the Ibsen diet the jaws and teeth of twenty-nine postnatal animals and twenty-seven embryos, whose mothers had been on the Ibsen diet throughout their lives, were examined:

Postnatal animals. Four of the postnatal guinea pigs were selected as parallels of the four animals of the negative controls and the five receiving orange juice which were more than 2 years old. These thirteen animals had received the same kind of food and had had similar treatment. Twenty of the animals on the Ibsen diet ranged in age from 1 day to 3 years. The ages of five of the animals were unknown, but they were adults and were considered old. The time on the diet varied from 14 to 75 days.

There were no soft spots at the base of any of the cheek teeth of the positive controls. In one animal a part of the alveolar bone was not so firm as the others but it was in no sense crumbly like the negative controls.

Embryos. The number of days after copulation is considered as the age of the embryo. The teeth and jaws of twenty-seven embryos whose mothers had been on the Ibsen diet during their entire life, were examined. These embryos were of twenty-one different litters. The age of the embryos ranged from 44 days to 67 days. Younger embryos were not examined since it is at 44 days the jaw bone and the dentine have become ossified (Harman and Smith, '36). With two exceptions both the areas at the bases of the cheek teeth and the alveolar bones were firm. In two embryos, 55 days and 56 days, respectively, the areas at the base of the third and fourth cheek teeth were not so firm as in the other embryos but they were not crumbly like the negative controls.

DISCUSSION

In the foregoing experiment attention was centered upon the variation of the amount of vitamin C in the diet. An attempt was made to have the other factors as nearly alike as possible in the parallel groups. Animals of about the

same ages which had been raised under similar circumstances were started on the different diets at the same time. When available, animals from the same litter were used. Animals of different ages from the newly born to those which were aged formed a part of each group so that the effect of age as a factor could be considered.

The results of this investigation agree with Howe ('20, '21, and '23), Höjer ('26) and Key and Elphick ('31) in that there is alveolar resorption. However, we have found that resorption of the bone of the jaw extends farther than merely the alveoli. In many instances the outer edges of the mandible is a crumbly mass which may be crushed under very little pressure. Contrary to the report of Fish and Harris ('34) we have found that when no vitamin C was added to the diet morphological changes have taken place in the bones of the jaws in the alveolar areas of the mandible and at the base of one or more of the cheek teeth even in animals which were old and which had been on the basal diet for only 28 days. There was not a single exception to this phenomenon. This condition could scarcely be due to the age of the animal since the ages varied from 20 days to 3 years and 3 months. Neither could it be due to the length of the time on the diet since there were the soft areas at the bases of the cheek teeth in one which had been on the diet only 26 days.

The jaw at the base of the two posterior cheek teeth is the first affected. All of the animals on the vitamin C free diet for more than 28 days had a crumbly alveolar area while those which had been deprived of vitamin C in the diet for only 28 days or less were not affected so extensively.

When this same diet was supplemented by 3 ml. of fresh orange juice per 300 gm. body weight there was scarcely a trace of resorption, although in four instances the animals were pregnant during the experiment. No indication of resorption was found in those animals which received a supplement of greens ad libitum. Animals fed the Ibsen diet had firm, strong jaw bones.

CONCLUSION

The data from this investigation show that:

1. In guinea pigs on the Sherman, LaMer, Campbell vitamin C free diet without supplement, there was resorption of bone at the bases of the cheek teeth and along the edge of the alveolar area. This resorption occurred without respect to the age of the animal.

2. Four weeks on the diet was sufficient time to produce a considerable amount of resorption.

3. The addition of 3 ml. of orange juice per 300 gm. body weight gave almost complete protection against resorption.

4. Guinea pigs fed the vitamin C free diet supplemented with greens ad libitum, those on the adequate Ibsen diet and embryos from mothers on the Ibsen diet had firm and strong jaw bones.

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THE UTILIZATION OF ENERGY PRODUCING NUTRI- MENT AND PROTEIN AS AFFECTED BY THE PLANE OF PROTEIN INTAKE ¹

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FIVE FIGURES

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The investigation to be discussed is a continuation of two earlier experiments on the same subject, the results of which were presented by Forbes, Swift, Black and Kahlenberg, in brief in 1935, and in full in this journal also in 1935.

In these earlier experiments equicaloric diets containing 10, 15, 20 and 25% of protein were compared in growth, metabolism and body analysis experiments in which twenty-four rats were used as subjects at each of the four planes of protein intake, with quadruplet, litter mate food control. The effects of the progressively greater protein contents of the diets were as follows:

Increase in gain in body weight, at decreased cost in terms of dry matter of food; increase in efficiency of digestion and retention of protein and of energy-producing nutriment; increase in urinary nitrogen at an increasing rate; increase in protein of the body at a decreasing rate; increase in outgo of energy in the urine coincident with decrease in fecal energy, the metabolizable energy remaining practically constant; diminished efficiency in the utilization of food nitrogen; no regular change in amount of fat gained, but usually a decrease in fat gained in proportion to protein gained.

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The progressively greater protein contents of the equi-caloric diets, having the effect to improve their nutritive balances, were accompanied by no change in the basal heat production per unit of computed surface area, but by diminished total heat production of the animals, as they lived under normal conditions of freedom of activity.

An investigation, in part of similar significance to the one by Forbes and associates, was conducted by Hamilton ('35, '37); and another study in the same general field was reported, in a preliminary way, by Hogan, Johnson and Ashworth ('35), and later, in full, by Johnson, Hogan and Ashworth ('36).

Hamilton commented briefly on his study ('35) while it was still in progress, and later ('37) published an abstract of the complete account of the investigation, the full report having as yet not come to publication.

Hamilton's first reference to results obtained ('35) was that "sufficient data have been obtained to warrant the statement that as the percentage of protein in the diet increases from 4 to 16 per cent, the thermogenic effect decreases," and this same statement was repeated, in effect, in the later abstract of the complete dissertation. It is a fact, however, that the data presented in the abstract referred to do not warrant the above quotation, without qualification, since, as the protein content of the diets increased from 4 to 8%, the thermogenic effect also increased, from 372 calories to 402 calories per gram of the diet.

The term 'thermogenic effects' was used to signify heat production in excess of the basal energy metabolism, and this quota was measured accordingly.

Some of the more important of Hamilton's conclusions are the following:

Increasing the percentage of protein from 4 to 16 per cent in the diet of growing animals increases the growth-promoting value of the diet. Diets containing between 16 and 30 per cent protein are of equal growth-promoting value, and as the protein increases above 30 per cent, the growth-promoting value decreases.

The voluntary 'running' activity of rats is unaffected by the protein content of the diet, until an excess (54 per cent whole egg protein) percentage is present, when the activity is decreased.

The net energy value of diets for growth plus maintenance increases as the percentage of protein increases up to about 16, remains practically constant throughout the range of protein percentages over which the diets are equally well balanced, and then decreases rapidly in diets containing percentages of protein in excess of that of a well-balanced diet (perhaps more than 30 per cent). The explanation of the higher net energy value of well-balanced diets, as compared with diets unbalanced either because of an insufficiency or because of an excess of protein, lies in the smaller thermogenic effects of the well-balanced diets.

The percentages of metabolizable energy used for basal energy expenditures or for total voluntary activity are unaffected by the percentage of protein in the diet.

A basis for the close interpretation of these findings, however, does not exist, since the method of compounding the diets, involving the direct quantitative substitution of protein for starch, changed not only the protein but also the gross and the metabolizable energy of the diets; and since the determination of 'thermogenic effects,' as increases of heat production above the basal energy metabolism, yielded extremely divergent values (135 to 625 calories), because of the different sparing effects of the diets on the katabolism of body substance of the rats in their initial basal status.

In other words these 'thermogenic effects' were of mixed nutritive origin and significance.

In the study of Johnson, Hogan and Ashworth ('36), two planes of protein intake, 10% and about 25%, were compared. The authors concluded, in part, that

growth was more rapid on the reasonably high protein diets than on like diets inadequate in protein content. These differences were not due to differences in energy lost in the excreta, in the total heat lost, or in the energy gains, but were due to differences in the kind of nutrients stored. The animals on the high protein diets stored more water, protein, and ash than their pair-mates, and thus stored less energy

per unit gain, while those on the low protein diets stored more fat than their pair-mates, and thus stored more energy per unit gain. The net utilization of energy for body gain by all animals was the same.

As with Hamilton's study, the plane of energy intake was not the same for the animals which received the two diets differing in protein content.

On the basis of the results obtained with the animals designated group 4, which afford the best opportunity for comparison, the animals on the high-protein diet received 6.7% more gross energy, and 5.7% more metabolizable energy, and they produced 3.7% more heat and 5.4% more energy gain than did those which received the low protein diet.

PLAN OF EXPERIMENT

The present experiment, like the two earlier ones by Forbes and associates, was a 70-day growth and metabolism investigation, employing the body-balance method, and the open circuit, Haldane, respiration procedure, the subjects being twelve quadruplets of albino rats (six each of males and of females) from the same number of litters, fed with quadruplet, litter mate food control.

In both the earlier and the present studies the problem, the methods of procedure, and the whole experimental set-up were the same, except that, whereas, in the two earlier experiments, the protein contents of the diets were 10, 15, 20 and 25%, respectively, in the present study they were 25, 30, 35 and 45%, respectively. The purpose of the present study was, obviously, to extend the evidence derived from the earlier studies in such manner that the entire series would cover the range of variation of protein contents of the diets from 10% to 45%—that is, from moderate deficiency to great excess of this nutrient.

Four diets were compared at once, with one rat from each quadruplet on each diet, there being as many individuals on a treatment, therefore, as there were quadruplets.

The diets having been compounded to be exactly equicaloric, but differing in protein content, and the initial weights of the animals of each quadruplet being the same, the food was given to the individuals of each quadruplet in equicaloric quantities, determined, for each quadruplet, by the individual animal which ate the least food.

The quantities consumed within quadruplets, therefore, were identical, though the food consumption was unlike for the different quadruplets.

This method of food assignment was designed to be as nearly equitable as practicable, but in the course of its use it becomes somewhat inequitable to those individual animals that have received the more efficient diets, since these favored subjects will have made the larger growth, and will be receiving less food in addition to their maintenance requirements than will those which have received the less efficient diets.

This situation prevents the full expression of the superiority of the better diets, and, therefore, has the effect to render the observed less than the true differences between the diets.

This error, however, is conservative in effect since it minimizes rather than exaggerates the differences in the results obtained.

In view of the method of food assignment employed, which resulted in equal energy intake within but different energy intake among quadruplets, and the observation made that the methods of use of food energy and protein were much affected by the food intake within equal-food-protein groups, the data derived from the quadruplets of rats which ate essentially the same quantity of food in the three experiments were segregated, both in the tables and the graphs, and were given special consideration as the most significant results of the investigation.

Course of the experiment

The investigation progressed, from beginning to end, with remarkable regularity, and without noteworthy incident or departure from plan.

As each quadruplet of rats was given increasing quantities of feed, from day to day, until one rat of the four failed to eat the entire quantity, the results afford a basis for conclusion as to the acceptability, to the rat, of the four diets, of different protein contents.

The numbers of such refusals, by the rats which received the diets of different protein contents, were as follows:

<i>Per cent protein in diets</i>	<i>Food refusals</i>	
	<i>First 3 weeks</i>	<i>Last 4 weeks</i>
25	59	81
30	66	111
35	33	48
45	152	65

These data show that the rats accepted the 35% protein diet most readily, and the 45% protein diet least readily, during the first 3 weeks, but that this difference existed only to an extent of doubtful significance during the last 4 weeks of the experiment. The order of the total number of refusals, for the 7 weeks, however, was as during the first 3 weeks. No especial importance is ascribed to these data; but it seems to be a fact that the 35% protein diet was the most acceptable to the animals. This response should be regarded more as an expression of final nutritive effect than of palatability.

Composition of diets

The percentage composition of the four diets was as in table 1.

It will be observed that these diets contained only 2.0% of butterfat, as a constant constituent, instead of 10.0%, as in the experiments discussed in the earlier paper; while the Crisco varied in percentage between 2.746 and 9.128, instead of between 0.0 and 5.102, as in the earlier experiments.

These differences came about as incidents in the computation of the four diets, each to be of the same energy value, but to contain the larger proportions of protein than as in the diets fed in the earlier experiments.

Then, the vitamin A content was safeguarded by the inclusion of carotene, in oil, as explained in the note below table 1. The gross energy value of these four diets was 4696 calories per gram.

TABLE 1
Composition of diets

	NO. 1	NO. 2	NO. 3	NO. 4
	%	%	%	%
Cellu-flour	4.000	4.000	4.000	4.000
O. and M. salt mixture	4.000	4.000	4.000	4.000
Na Cl	1.000	1.000	1.000	1.000
Yeast ¹	6.000	6.000	6.000	6.000
Butterfat	2.000	2.000	2.000	2.000
Dextrin	49.374	45.360	41.345	33.316
Casein	24.498	30.108	35.718	46.938
Crisco	9.128	7.532	5.937	2.746

¹ The yeast was a mixture of 5 parts brewer's yeast and 1 part irradiated yeast.

NOTE: Carotene, in oil, was added to each diet, in the proportion of 2.5 gm. to each 7500 gm. of other constituents. This supplied 1.4 U.S.P. units of vitamin-A-equivalent per gram of food.

TABLE 2
Food eaten, and average quantities, character and gross efficiency of gains in weight

PLANE OF PROTEIN INTAKE	FOOD EATEN (DRY MATTER)	GAIN IN BODY WEIGHT ¹	FOOD (DRY MATTER) PER GRAM BODY GAIN	NITROGEN OF BODY GAIN	FAT GAINED	FAT GAINED PER GRAM N GAINED
% protein	gm.	gm.	gm.	gm.	gm.	gm.
25	451	116.70	3.9 ± 0.11	3.79	21.7	5.7 ± 0.25
30	451	112.87	4.0 ± 0.13	3.67	20.5	5.6 ± 0.26
35	451	112.34	4.0 ± 0.11	3.70	19.6	5.3 ± 0.24
45	451	104.76	4.3 ± 0.13	3.45	17.7	5.1 ± 0.25

¹ Contents of alimentary tract removed.

NOTE: Each datum is an average representing twelve animals on a continuous metabolism experiment during 10 weeks.

Body gains

Referring to table 2, and to figure 1, it is clear that, with the same food intake, the progressively greater protein contents of the diets above 25% resulted in diminished gain in body weight. Such greater protein contents of diets as did not serve to improve the efficiency with which the food protein

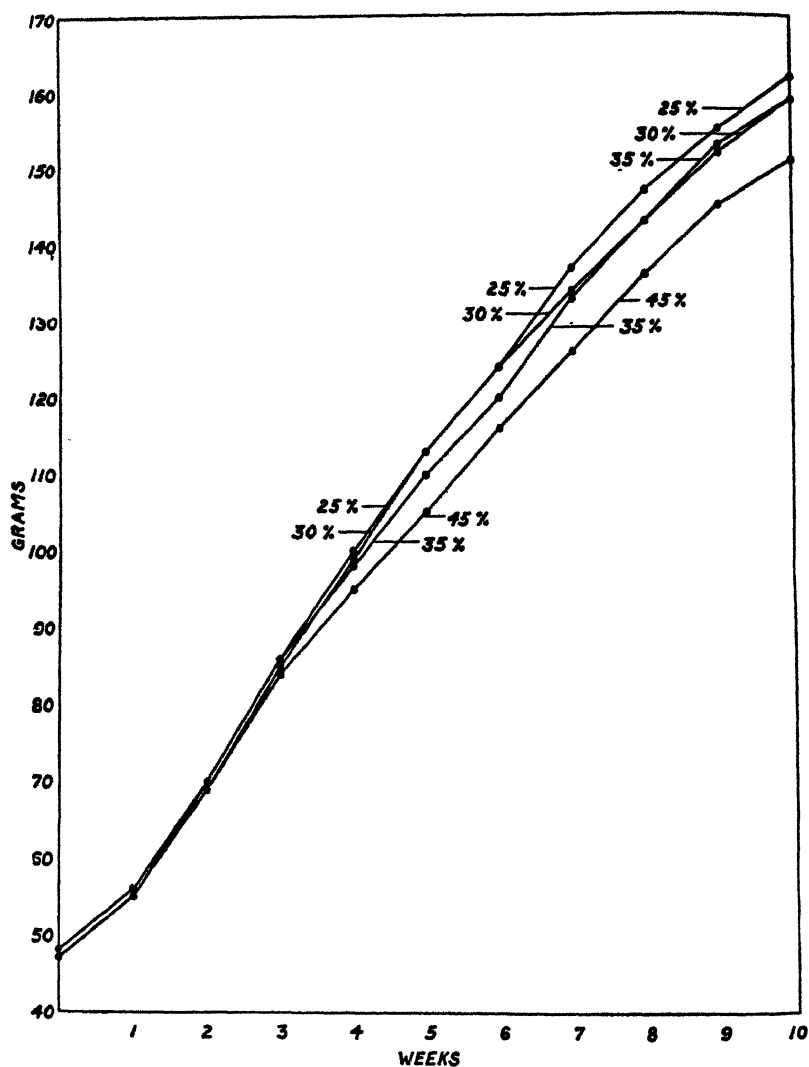


Fig.1 Representing growth of albino rats during 10 weeks. Each curve represents twelve rats, each such group having received the same average energy intake, but a different percentage of protein in the diet.

was retained were definitely disadvantageous, because of the lower metabolizability of protein than of non-nitrogenous organic nutrients.

The odds that the average gain in body weight from the 25% protein diet was greater than that from the 45% protein diet were more than 10,000 to 1.

TABLE 3

Distribution of average total food energy as affected by the plane of protein intake

PLANE OF PROTEIN INTAKE	FOOD ENERGY	FECES	DIGESTED	URINE	METABOLIZED	BODY GAIN	BODY GAIN AS PROTEIN	BODY GAIN AS FAT	HEAT
Representing all animals fed, experiments 1 and 2									
%	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.	Cal.
10	2119.8	181.4	1938.4	42.9	1895.5	225.3	80.9	144.4	1670.2
15	2119.8	170.0	1949.8	54.4	1895.4	261.0	122.5	138.5	1634.4
20	2119.8	150.2	1969.6	71.6	1898.0	281.5	134.9	146.6	1616.5
25	2119.8	140.6	1979.2	91.3	1887.9	285.9	139.0	146.9	1602.0
Representing all animals fed, experiment 3									
25	2261.3	161.2	2100.1	99.4	2000.7	331.9	129.1	202.8	1668.8
30	2261.3	156.4	2104.9	120.7	1984.2	319.5	126.8	192.7	1664.8
35	2261.3	149.4	2111.9	142.4	1969.5	311.2	127.3	183.9	1658.3
45	2261.3	140.1	2121.2	187.2	1934.0	284.5	118.5	166.0	1649.5
Representing animals selected for uniform food intake, experiments 1 and 2									
10	2137.1	182.6	1954.5	43.6	1910.9	212.3	77.2	135.1	1698.6
15	2137.1	166.1	1971.0	54.5	1916.5	257.1	118.9	138.2	1659.4
20	2137.1	150.7	1986.4	72.2	1914.2	274.8	129.4	145.4	1639.4
25	2137.1	140.8	1996.3	91.9	1904.4	297.6	138.5	159.1	1606.8
Representing animals selected for uniform food intake, experiment 3									
25	2141.0	151.2	1989.8	95.9	1893.9	304.6	117.6	187.0	1589.3
30	2141.0	151.2	1989.8	115.4	1874.4	278.1	115.5	162.6	1596.3
35	2141.0	143.1	1997.9	136.0	1861.9	282.0	114.2	167.8	1579.9
45	2141.0	131.8	2009.2	178.2	1831.0	255.0	111.2	143.8	1576.0

It is also shown, in table 2, that progressively greater protein contents of the equicaloric diets, from 25 to 45%, caused decreased gains of nitrogen and of fat, but a study of the proportion of fat gained to nitrogen gained, on the basis of the data in this table and those presented in table 3, did not reveal consistent effects of the plane of protein intake on the composition of the body gains.

The average ratios of protein gained to water gained, for the groups which received the diets differing in protein content, were identical. The data are not presented.

Distribution of energy intake

In the remainder of the present paper the data from the experiments of the earlier study are combined, for purposes of discussion, with the new data from the third experiment, in order to make a unified presentation of the results of the entire investigation, covering the range of variation between 10% and 45% in the protein of the diets.

In table 3 is represented the distribution of the total quantities of food energy received by the rats as affected by the plane of protein intake, this table being divided into four sections, the first representing all animals fed in experiments nos. 1 and 2 (the published experiments); the second, all animals fed in experiment no. 3 (the new experiment); the third, a selection of quadruplets of animals which received the same quantity of food, in experiments nos. 1 and 2; and the fourth, a similar group of quadruplets from experiment no. 3, selected for essentially the same uniform food intake as that representing experiments nos. 1 and 2, in the third section of the table.

These data for selected animals, given in the third and fourth sections of the table, represent five quadruplets of rats from the first, and seven from the second experiment, the average energy intake during the 10 weeks in both groups being 2137 Calories, and six quadruplets from the third experiment, the average energy intake of which was 2141 Calories.

The data comprising the first two sections of table 3 are graphically presented in figure 2, and those in the last two sections are represented in figure 3.

The breaks in the continuity of the curves in figure 2 result mainly from differences in the average plane of nutrition, but apparently also to a slight extent from the differences in the composition of the diets, which have been explained.

These breaks, therefore, are largely eliminated by the selection of quadruplets of animals for uniform food intake, as represented in figure 3; but this selection did not serve perfectly to smooth out the curves representing some of the data,

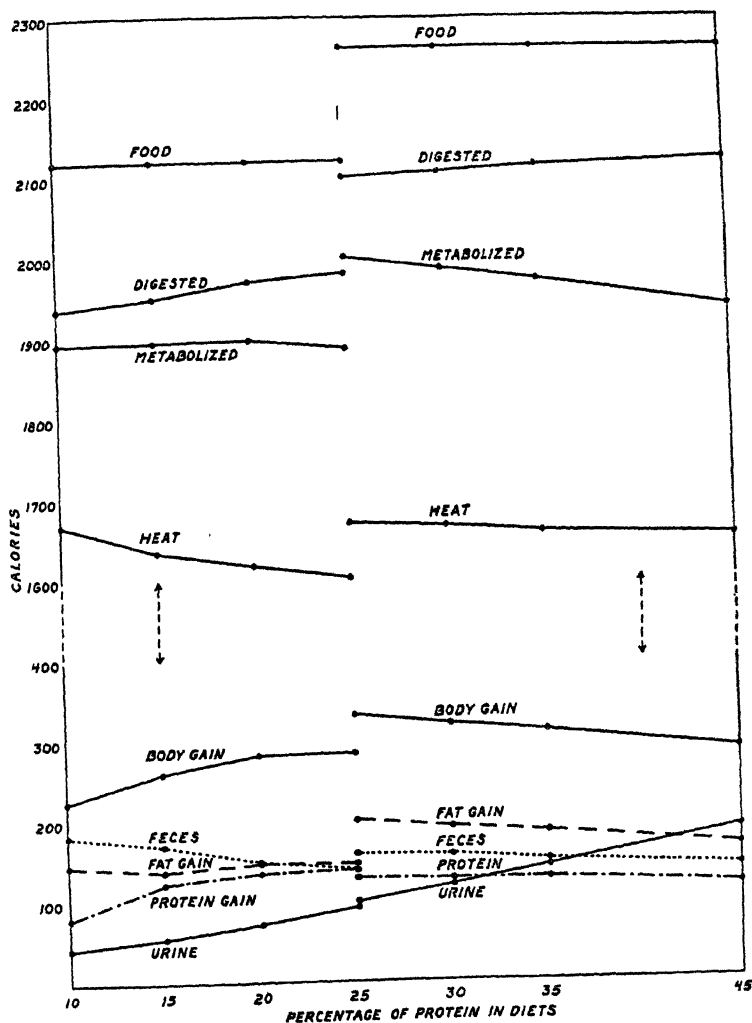


Fig. 2 Representing the distribution of the average food energy of all animals fed, as affected by the plane of protein intake.

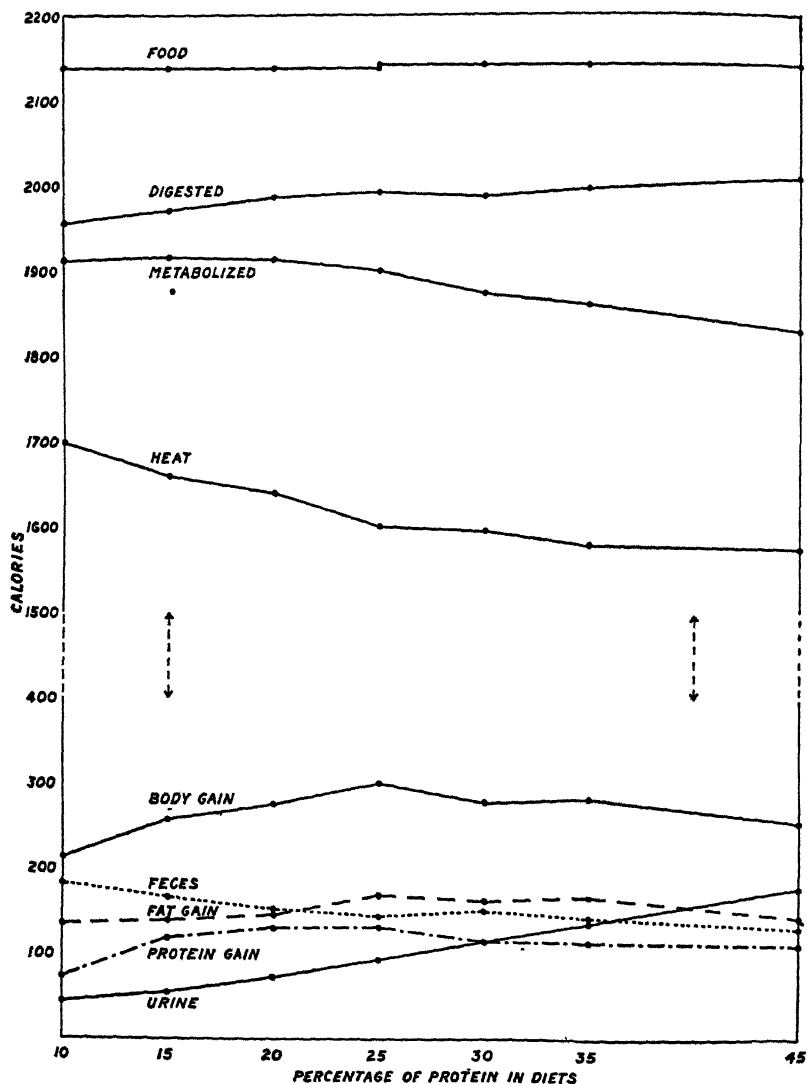


Fig. 3 Representing the distribution of the average food energy of animals selected for uniform food consumption, as this distribution was affected by the plane of protein intake.

especially those relating to the fat gain and the protein gain, since the composition of the body increase of rats is much affected by the individuality of the animal, and since the selected data represent comparatively few individuals—only half of the total number of animals fed.

Referring to figure 3, which is considered more significant than figure 2, the curves representing fecal energy and digested energy show that with progressively greater protein contents of the diets there were increasing quantities of food energy digested.

Digestibility, as here represented, was 'apparent' digestibility, that is, without separate consideration of fecal nitrogen of metabolic origin.

With diets containing 10, 15, 20 and 25% protein the progressively smaller proportions of fecal energy approximately balanced the progressively larger proportions of urinary energy, the metabolizable energy being virtually the same for all diets, and the increasing quantities of energy utilized for body gain balancing the diminishing heat production.

With more than 25% of protein in the diets the greater quantities of urinary energy more than counterbalanced the smaller quantities of fecal energy—the quantities of both metabolizable energy and energy of body gain diminishing.

With these diets containing 25 to 45% of protein the rapidity of the decrease in the quantities of the resulting products, corresponding to the progressively greater proportions of protein, was first in metabolizable energy, second in energy of body gain, and third in heat production.

The values for metabolizable energy were computed as gross food energy minus the energy of the feces and urine, without correction for the fraction of the body gain which is non-metabolizable—in the sense of not being available for heat production. In some relations, and for some purposes, such a correction is made in studies conducted at this institute.

As indicative of the validity of the observations of gain of energy, the odds that the gain from 25% protein diet was greater than that from the 45% protein diet were 5000 to 1.

The outgo of energy in the form of heat (computed as the gross energy of the food minus the energy of the excreta and of the body gain) diminished, with a diminishing rate of decrease, as the protein contents of the diets increased from 10 to 45%.

The heat which represented voluntary activity was not separated from that which represented energy expense of food utilization, but the basal heat production seems to have been unaffected by the plane of protein intake. The curve of heat production can best be interpreted, therefore, in general terms, simply as one of the effects of the differences in nutritive balance, the decrease in heat from diets containing 10, 15, 20 and 25% protein representing mainly improved nutritive balance, and the slight continuing decrease of heat from diets containing 30, 35 and 45% protein representing mainly diminished metabolizability (due, in this case, to increased urinary energy) of the diets.

The foregoing observation on the basal heat production in relation to the protein contents of the diets is based on determinations of this value as reported in the earlier paper, by Forbes and associates, and on further determinations made in connection with the present (third) experiment, these last-mentioned determinations having been made after the rats had been on experiment for 6 weeks.

The average values found in these last determinations, expressed as calories per computed square meters of body surface, per hour, were as follows: for the 25% protein rats, 32.9 Calories; for the 30% protein rats, 33.4 Calories; for the 35% protein rats, 34.0 Calories; and for the 45% protein rats, 33.7 Calories.

The calculated odds that the differences between these averages are statistically significant are as follows: 25 and 30% protein, 3:1; 25 and 35% protein, 293:1; 25 and 45% protein, 22:1; and 30 and 35% protein, 4:1. The difference between the values for the rats which received the diets containing 25 and 35% protein being only 3.25% of the smaller value, the significance of this difference seems highly questionable, in spite of the computed odds, especially because

of the indefiniteness of the so-called basal metabolism as a physiological quota.

The distribution of the food energy on a percentage basis is presented in table 4, these data being in harmony with the general trends observed in the corresponding data on the absolute basis.

The remarkable fact made clear by the evidence presented is that the heat production is as little affected as it was found to be by the plane of protein intake.

TABLE 4

Percentage distribution of average total food energy by animals selected for uniform intake of food energy

PLANE OF PROTEIN INTAKE	FECES	DIGESTED	URINE	METABO- LIZED	BODY GAIN	BODY GAIN AS PROTEIN	BODY GAIN AS FAT	HEAT
Experiments 1 and 2								
%	%	%	%	%	%	%	%	%
10	8.5	91.5	2.0	89.5	9.9	3.6	6.3	79.6
15	7.8	92.2	2.6	89.6	12.0	5.6	6.4	77.6
20	7.1	92.9	3.4	89.5	12.9	6.1	6.8	76.6
25	6.6	93.4	4.3	89.1	13.9	6.5	7.4	75.2
Experiment 3								
25	7.1	92.9	4.5	88.4	14.2	5.5	8.7	74.2
30	7.1	92.9	5.4	87.5	13.0	5.4	7.6	74.5
35	6.7	93.3	6.4	86.9	13.2	5.3	7.9	73.7
45	6.2	93.8	8.3	85.5	11.9	5.2	6.7	73.6

The significance of the values for heat production, so far as they affect net energy, is that net energy is not a characteristic function of individual nutrients, or of individual foodstuffs (except as these may be fed alone), but is a function of the entire diet—a point of view which was expressed by Forbes, in 1929, and which has since been discussed by Forbes, or by Forbes and associates, in numerous publications, but especially in papers cited as by Forbes, Braman, Kriss and Swift ('31), Forbes ('33 a, b) and Forbes, Braman, Kriss and Swift ('33).

Distribution of nitrogen intake

In table 5 are set forth the data for distribution of food nitrogen as affected by the plane of protein intake, these observations, for all animals fed, being graphically illustrated

TABLE 5

Distribution of average total food nitrogen as affected by the plane of protein intake

PLANE OF PROTEIN INTAKE	FOOD NITROGEN	FECES	DIGESTED	URINE	BODY GAIN
Representing all animals fed, experiments 1 and 2					
%	gm.	gm.	gm.	gm.	gm.
10	7.23	0.86	6.37	3.82	2.21
15	10.79	0.90	9.89	6.12	3.35
20	14.28	0.96	13.32	9.12	3.71
25	17.88	1.01	16.87	12.39	3.80
Representing all animals fed, experiment 3					
25	19.12	1.21	17.91	14.34	3.79
30	23.55	1.29	22.26	17.97	3.67
35	27.21	1.31	25.90	21.85	3.70
45	34.62	1.44	33.18	29.20	3.45
Representing animals selected for uniform food intake, experiments 1 and 2					
10	7.28	0.86	6.42	3.87	2.14
15	10.87	0.92	9.95	6.18	3.29
20	14.41	0.96	13.45	9.29	3.58
25	18.04	1.02	17.02	12.51	3.77
Representing animals selected for uniform food intake, experiment 3					
25	18.10	1.11	16.99	13.90	3.43
30	22.30	1.28	21.02	17.13	3.35
35	25.76	1.29	24.47	20.93	3.33
45	32.78	1.33	31.45	27.68	3.25
Exp's 1, 2, 3 Av. 25 (selected)	18.06	1.05	17.01	12.97	3.66

in figure 4; and for animals selected for uniform food consumption in figure 5.

An interesting arithmetical principle is exemplified, in this figure, by the nearly parallel lines representing food nitrogen and digested nitrogen, and by the nearly horizontal line representing fecal nitrogen.

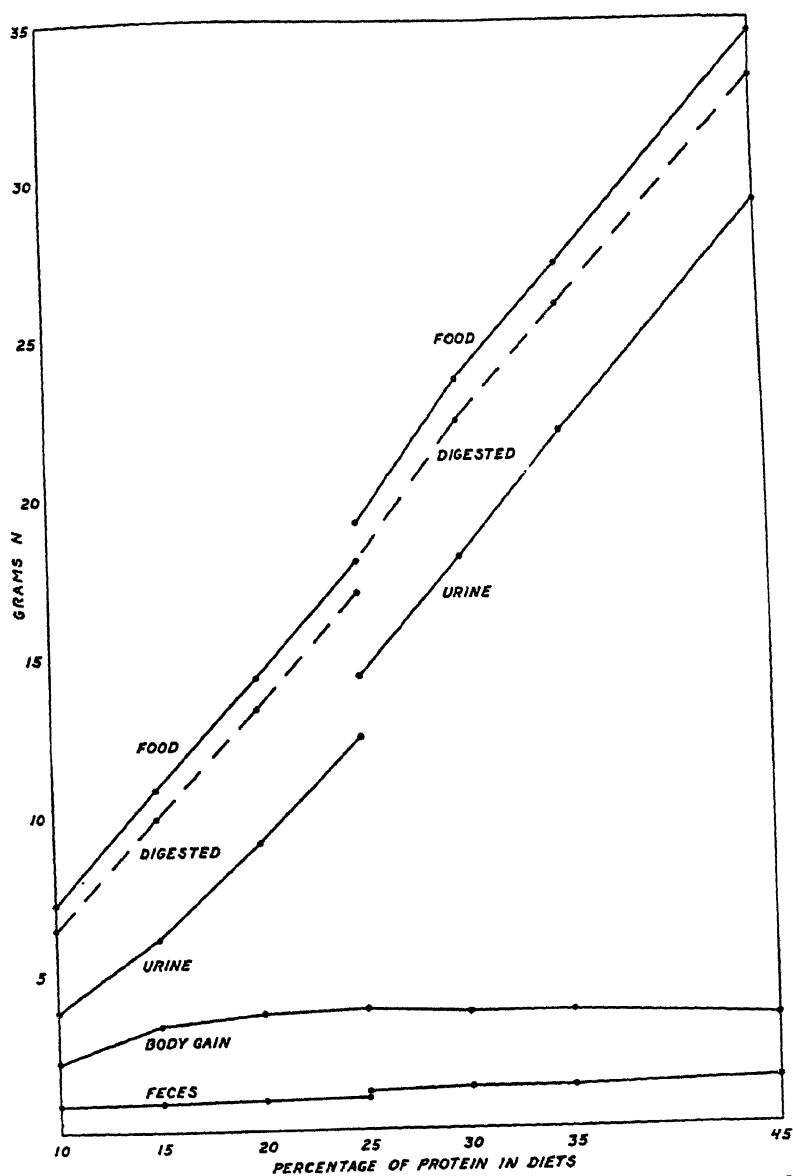


Fig. 4 Representing the distribution of the average food protein of all animals fed, as affected by the plane of protein intake.

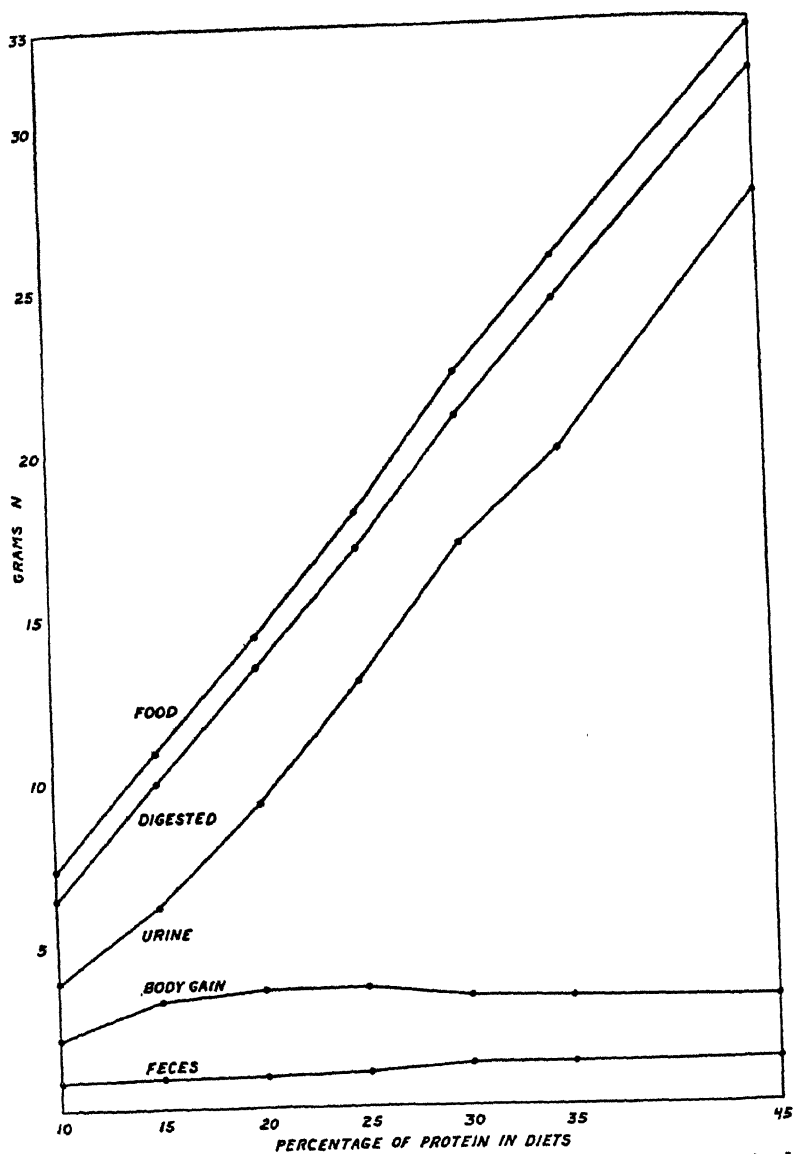


Fig. 5 Representing the distribution of the average food protein of animals selected for uniform food consumption, as this distribution was affected by the plane of protein intake.

The natural inference from a glance at these lines is that the digestibility of the food nitrogen increased at essentially the same rate as did the intake of food nitrogen, and, therefore, that the digestibility was not significantly affected by the plane of protein intake; but this would be in error, because the nearly constant outgo of nitrogen in the feces constituted a significantly diminishing proportion of the rapidly increasing nitrogen intake.

The substance of the matter is that the values given for digestibility were for apparent digestibility, and that the fecal nitrogen was so little affected by the plane of protein intake as to indicate that it (the fecal nitrogen) was nearly all of metabolic origin.

The sums of the values given for nitrogen of feces, urine and body gain, in this table, do not agree exactly with the values given for food nitrogen, since these values were all determined directly, and independently. These discrepancies represent the resultants of the slight, inevitable errors of determination of the four above-mentioned nitrogen values. Among these factors of error a visible one is contributed by shed hair, which is not accounted for, and a second possible factor of error is loss of ammonia from the urine between the time it is voided and the time it is collected.

A significant view of this consideration of the effect of the plane of protein intake on the distribution of food nitrogen is presented in table 6, in which it is shown that corresponding to progressively greater proportions of protein in the diets a diminishing percentage of the intake was eliminated in the feces; an increasing percentage was apparently digestible; an increasing percentage was eliminated in the urine; and a diminishing percentage was utilized for body increase.

The effect of the plane of protein intake on the distribution of the urinary constituents in the whole series of experiments is revealed in table 7. In the first and second experiments, in which the percentages of protein in the diets were 10, 15, 20 and 25, respectively, the Calories per gram of nitrogen in the urine diminished from 10.6 to 7.4; and in the third experiment, with percentages of protein in the diets of 25, 30, 35

and 45, respectively, and a plane of food intake slightly higher than in the first two experiments, the Calories per gram of urinary nitrogen diminished from 6.9 to 6.4.

The reason that the values, in table 7, for the grams of urinary nitrogen resulting from the 10% and the 15% protein rations, differ slightly from the corresponding values in table

TABLE 6

Percentage distribution of average total food nitrogen by animals selected for uniform intake of food energy

PLANE OF PROTEIN INTAKE	FECES	DIGESTED	URINE	BODY GAIN
Experiments 1 and 2				
%	%	%	%	%
10	11.8	88.2	53.2	29.4
15	8.5	91.5	56.9	30.3
20	6.7	93.3	64.5	24.8
25	5.7	94.3	69.3	20.9
Experiment 3				
25	6.1	93.9	76.8	19.0
30	5.7	94.3	76.8	15.0
35	5.0	95.0	81.3	12.9
45	4.1	95.9	84.4	9.9

TABLE 7

Relationship of nitrogen and energy in urine with different levels of protein in the diet

PLANE OF PROTEIN INTAKE	NITROGEN OF URINE	ENERGY OF URINE	CALORIES PER GRAM OF NITROGEN IN URINE	PLANE OF PROTEIN INTAKE	NITROGEN OF URINE	ENERGY OF URINE	CALORIES PER GRAM OF NITROGEN IN URINE
%	gm.	Cal.		%	gm.	Cal.	
10	3.88	41.0	10.6	25	14.34	99.4	6.9
15	6.18	54.9	8.9	30	17.97	120.7	6.7
20	9.11	71.5	7.8	35	21.85	142.4	6.5
25	12.39	91.3	7.4	45	29.20	187.2	6.4

5 is that during the use of these data, from the earlier paper on the same subject as the present one, errors—apparently of transposition—were discovered in two individual data contributing to the above-mentioned averages. These two erroneous values were excluded from the averages given in table 7.

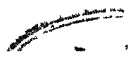
The significance of these data is that with progressively greater protein contents of the diets there is a decrease in the proportion of nitrogenous compounds of endogenous origin, and an increase in the proportion of urea, in the urine. The Calories per gram of nitrogen in urea being 5.47, and the decrease in Calories per gram of nitrogen, as the percentage of protein in the diet increased from 35 to 45 being only from 6.5 to 6.4, it is obvious that further possible increase in the protein of the diet could produce but little further decrease in the Calories per gram of nitrogen in the urine.

SUMMARY

Corresponding to progressively greater protein contents of equicaloric diets, from 10 to 45%, were increased digestibility and decreased metabolizability of food energy; decrease in heat production at a diminishing rate of decrease; increase in energy of urine; increase in gain in body weight and in energy of body gain until the optimum proportion of protein in the diet was reached, and, with further increase in protein, slight decreases in rate of gain in weight, energy, nitrogen and fat, and in fat gained per gram of nitrogen gained.

The nutritive balance of the diets as sources of energy was obviously improved, corresponding to their progressively greater protein contents from 10% to 25%, as evidenced by marked increase in energy of body gain; and approximately equal decrease in heat production—the metabolized energy remaining virtually unchanged.

Corresponding to further increase in the protein contents of the diets from 25 to 45%, the nutritive balances of the diets as sources of energy were slightly impaired, as evidenced by appreciable decrease in the quantity of energy utilized for body gain, due to more rapid decrease in metabolizable energy than in heat production—the decrease in metabolizable energy resulting from an increase in energy of urine which exceeded the slight decrease in energy of feces.



Corresponding to progressively greater protein contents of the diets from 10 to 45%, there was a slight decrease in the proportion of food nitrogen appearing in the feces; a considerable increase in the proportion appearing in the urine; and first a marked increase, followed by a marked decrease, in the proportion utilized for body gain.

The plane of protein intake did not materially affect the basal energy metabolism.

The results tend to sustain the idea that the specific dynamic effects of protein, carbohydrate and fat, as their relative values are ordinarily understood, do not apply in relation to the mixed diets of nutritive practice; and the idea that neither individual nutrients nor individual feeding stuffs express their maximum, normal, nutritive values except as components of complete diets.

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STUDIES ON THE EFFECTS OF A BOVINE BLINDNESS-PRODUCING RATION UPON RABBITS ¹

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THREE FIGURES

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Blindness of cattle caused from stenosis of the optic foramen seems to arise from nutritional sources. This type of blindness has been reported by Huffman ('29), Walker ('36) and others. Huffman and co-workers associated the blindness with poor quality roughage and found that cod liver oil was effective in its prevention, although Moore, Huffman and Duncan ('35) found that 10 to 20 mg. of carotene daily failed to protect calves. A somewhat similar type of blindness has been found to accompany the feeding of cottonseed meal and poor roughage. Reed, Huffman and Addington ('28) obtained it on rations when cottonseed, or linseed meal, was fed with wheat straw as the roughage. Halverson and Sherwood ('30) suggested a vitamin A deficiency but were unable to entirely supplement cottonseed meal rations for dairy cows by the use of 27% alfalfa hay. Bechdel and Skaggs ('35) were unable to arrest the blindness by the administration of large doses of carotene. Gallup, Kuhlman and Weaver ('36) suggested that some nutritive factor other than vitamin A or D was responsible since cod liver oil was superior to carotene or tomato juice and vitamin D. de Schweinitz ('32) considers the problem to be a genetic factor. Huffman ('29)

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and Walker ('36) have shown that vitamin D is not involved. The latter author states that "vitamin A cannot be entirely ruled out," although his evidence suggested that some other factor might be responsible for the blindness.

Since considerable uncertainty exists as to the cause of the stenosis of the optic foramen an attempt to produce and study it with laboratory animals was undertaken.

EXPERIMENTAL

A series of experiments was made. In a preliminary experiment no. 361 an attempt was made to produce in the rabbit this blindness which has been referred to as atypical blindness. A second experiment no. 362 was planned to study the efficiency of various methods of supplying carotene as a source of vitamin A, while a third experiment no. 363 was planned to re-check the efficacy of carotene for preventing blindness on the Walker ration and to control the effects of the carotene carrier.

The basal ration used in this series of experiments was one that was used by Walker ('36) in producing optic foramen stenosis in calves. It was composed of white corn, 22 parts; linseed oil meal, 23 parts; wheat middlings, 11.5 parts; oat mill feed, 40 parts; ground limestone, 3.0 parts; iodized salt, 0.5 parts, and irradiated yeast, 0.1 part.

Experiments were made with both guinea pigs and rabbits and identical results were obtained with each species. However, the routine difficulties of supplying ascorbic acid to care for the vitamin C requirements and the eccentricity of the guinea pig led to its elimination for these studies and only the results with rabbits are reported here. Four young rabbits were used in experiment no. 361. All received the basal ration. One received in addition 150 micrograms of (SMA) carotene daily per kilogram of body weight fed in Wesson oil as a carrier and the second received similarly 30 micrograms of carotene daily per kilogram of body weight. Twenty young rabbits were used in experiment no. 362 and divided into five lots as follows:

Lot I. Basal ration only.

Lot II. Basal ration plus 2% Wesson oil containing sufficient carotene to furnish 1.1 microgram of carotene per gram of feed.

Lot III. Basal ration plus a daily subcutaneous injection of carotene suspended in water. Dosage 44 micrograms per kilogram body weight. This dosage was adjusted as indicated later.

Lot IV. Basal ration plus 2% aerated cod liver oil.

Lot V. Basal ration plus 1.1 microgram carotene per gram of feed evaporated from an ether solution.

The aerated cod liver oil used in lot IV was prepared by the usual method used in this laboratory (Rupel et al., '33) for the purpose of destroying vitamin A.

In experiment no. 363 young rabbits were placed on the basal ration plus ample carotene for 1 week and then allotted to four lots of three animals each on the basal ration. Lots I and II received added carotene, I in the form of oral administration of carotene in Wesson oil at the rate of 50 micrograms per kilogram of body weight, and II was given the carotene by adding 2% Wesson oil carrying enough carotene to give 0.6 micrograms per gram of feed. Lots III and IV were on the basal ration only but in the latter 2% of unfortified Wesson oil was added.

The rabbits used in these experiments were weanlings of the American white, chinchilla or Himalayan breeds. The harshness of the ration caused some difficulty with the rabbits during the first weeks and considerable mortality was experienced. Animals of the same sex were used in experiments nos. 361 and 363. In experiment no. 362, one male and three females were placed in each lot.

RESULTS

This series of experiments gave consistently concordant results in the following respects: Stenosis of the optic foramen could not be produced in the rabbit, no doubt due to the anatomical arrangement of the skull. Blindness and ataxia developed in the rabbit on the unsupplemented ration with strikingly similar symptomology to that of the growing calves. Growth was retarded on the basal ration. Supplementary additions of carotene from the beginning of the experiment in doses of 50 or more micrograms per kilogram of body

weight completely prevented the development of the blindness and ataxia. This level permitted normal growth, health and vigor over an 8-month period, and it was adequate for pregnancy and gestation. The mode of administration of the carotene was without influence provided the dose was ample at all times. Wesson oil, aerated cod liver oil, or lactoflavin failed to prevent the disease. Once the syndrome was distinctly apparent doses of 35 or 70 micrograms of carotene (SMA Company crystalline carotene) per kilogram of body weight per day were ineffective in arresting or remedying the progress of the disease. The basal ration alone produced 100% mortality.

The symptoms shown by the rabbits developing this syndrome were strikingly similar in all respects to those produced in calves. They may begin as an involvement of locomotion, equilibrium, or sight. In approximately 50% of the cases ataxia and loss of equilibrium developed concurrently with or before the eye symptoms began. Usually, however, the first symptoms appeared in the eye with a fleeting or persistent ophthalmia, hyperemia, and congestion, followed very soon by an erosive dry keratitis progressing rapidly from Bitot's spots to opacity and blindness. The keratitis observed was the interstitial type which gave a ground glass appearance to the cornea as shown in figure 1. The light reflex was sluggish and somewhat disturbed. This condition was accompanied by progressive ataxia and loss of equilibrium when posture was disturbed quickly. This was observed most frequently when the animals were lifted free of the floor. Convulsive efforts were made in an attempt to regain 'position' although no definitely clonic seizures were seen. Infrequent stiffness and partial paralysis of the legs occurred, most frequently noted in the front quarters. The normal position of the head was often lost. Persistent diarrhea and salivation accompanied the disorder, particularly in the later stages.

Growth was retarded and in severe cases loss of weight occurred as shown in figure 2. The administration of 70

micrograms of carotene to the animals in experiment no. 361, lot III, failed to prevent loss of weight and other symptoms. Two per cent aerated cod liver oil was ineffective in maintaining normal growth and preventing loss of body weight. Likewise 2% Wesson oil was without potency unless fortified with carotene. A flavin concentrate given in large doses for

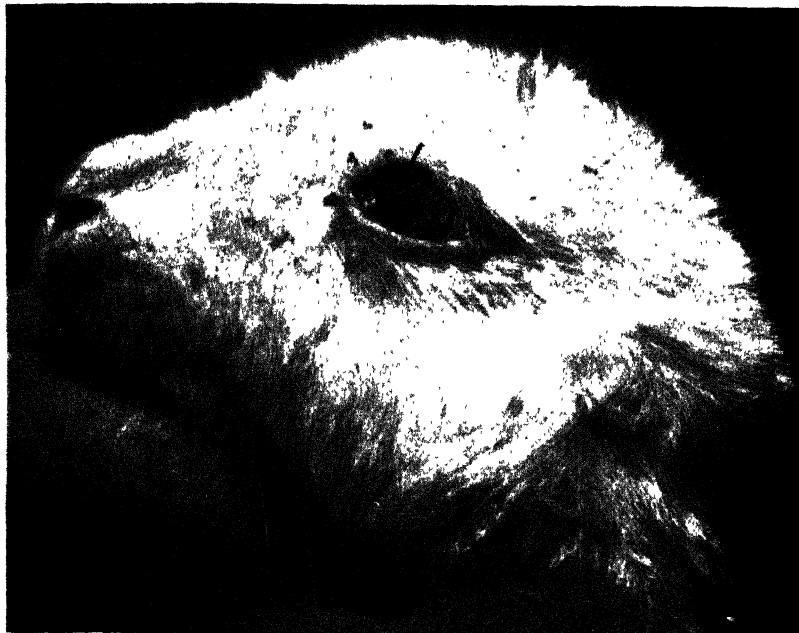


Fig. 1 A rabbit from experiment 363 lot III which received the basal ration only. Note the ground glass appearance of cornea.

a week had no beneficial effect. The daily injection of 44 micrograms of carotene suspended in water was ineffective for growth promotion and prevention of the development of the syndrome. At point A, figure 2, lot III, experiment no. 362, four massive doses (15,000 units) of vitamin A were given to one animal over a period of 8 days and another animal was given a daily injection of 300 γ of carotene for 1 week. Thereafter both animals were continued on a daily

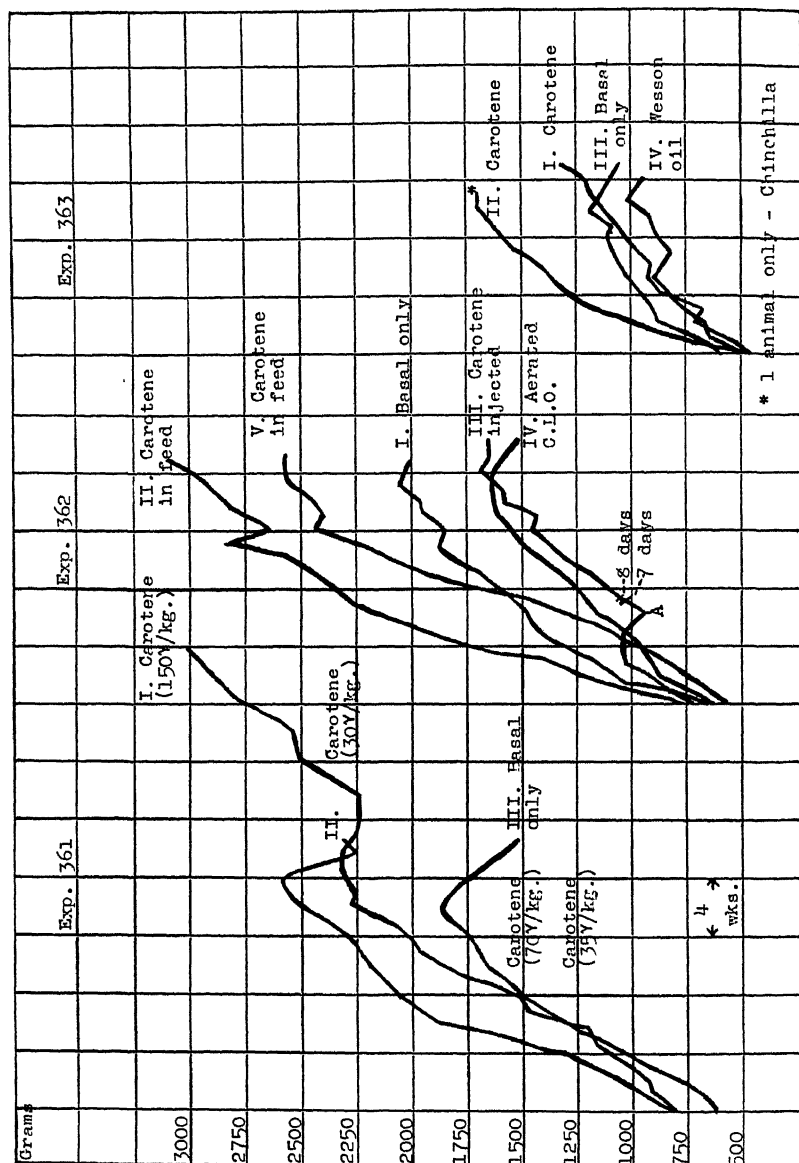


Fig. 2 Growth curves of rabbits on experimental diets.

injection of 100 γ per kilogram of body weight. Growth, which had stopped completely, was resumed in both cases although at a retarded rate. This was accompanied by a recession of symptoms and gradual return to normal.

Pathological analysis of the first experiment was not attempted. The animals in the other experiments were utilized for histological material and portions of the central and peripheral nervous system were taken for microscopic study as well as portions of the liver, spleen, kidney and suprarenals. Examination of the brachial plexus area, the optic and sciatic nerves, were made with the polarizing microscope and with appropriately fixed material by the rapid Marchi method of Geist ('35), Sudan III, silver, and H and E. In general the results obtained with the Marchi method, Sudan III, and the polarizing microscope gave concordant results on the peripheral nerves. The latter was found to be of little use in the study of the optic nerve and cord. A total of eight animals was studied.

Six of these showed kidney damage in the glomeruli and tubules. The changes in the kidney were early mild parenchymatous degeneration in the proximal convoluted tubules, a general tendency for connective tissue response with focal interstitial proliferation (fig. 3), inflammation and congestion with some oedema. Attention is called to the marked similarity of the histopathology to that reported by Reed and associates ('28). Metaplasia of the renal pelvis epithelium was observed in two cases. Five livers showed a reaction in the von Kupfer cells and hepatic congestion. In the former case there seemed to be sufficient hypertrophy to bring the von Kupfer cells into prominent relief in contrast to their usual inconspicuous place in the tissue.

Three cases had some involvement of the spleen. In the central nervous system no changes were observed in the brain itself. In one case a good section was obtained of the choroid plexus. The ependymal cells showed vacuolization and slight hypertrophy as distinct from the normal regularity of the structure. These histologic changes were similar to

those observed in chicks suffering from vitamin A deficiency (Phillips, '37). Myelin degeneration was found in the sciatic nerve of five animals and in the region of the brachial plexus in three. Slight alteration was noted in the optic nerve but its interpretation was doubtful. Three animals showed an alteration of the Purkinje cells of the cerebellum which was marked by atrophy, a homogenous cell change and areas of

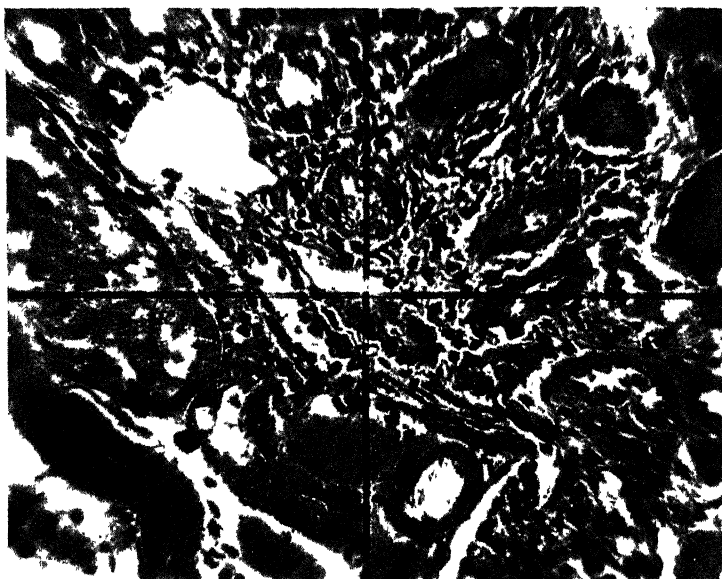


Fig. 3 Photomicrograph of an area of the kidney of a rabbit fed the basal ration only. Note the interstitial proliferation and parenchymatous degeneration. Magnification $\times 450$.

cell disappearance. In the areas where the Purkinje cells had disappeared, Bergman cells were conspicuous.

Pregnancy and gestation were obtained in experiment no. 362 where carotene was added to the ration. No evidence of reproductive activity was observed on the basal ration alone or with additions of 2% Wesson oil, 2% aerated cod liver oil, or where the carotene was injected. Attempts to carry the females through a lactation period were not made.

Examination of the optic foramina showed little if any change remotely suggesting stenosis. Frequently the lacrimal glands were inflamed and swollen. At least one instance of secondary inflammation involving the maxillary bone was observed, and exostosis was found in the floor of the orbit which was suggestive of possible changes which might be anticipated in severe infections. In most cases the molar teeth of these rabbits lacking adequate amounts of vitamin A were unevenly worn.

DISCUSSION

It is apparent from these experiments that blindness from stenosis of the optic foramen cannot be attained in rabbits by the use of the Walker diet. In all other respects the syndrome developing in the rabbit from this ration low in vitamin A is analogous to that of calves. The symptomatology, behavior, growth curves, and ultimate course of the disease were alike. The ration was shown by Walker ('36) to contain from 9 to 10 mg. of carotene per 100 pounds. It seems that small quantities of vitamin A such as this ration contains permit physiological activity of low grade in the slow growing species. Thus variable manifestations may occur. It is possible that heavy sinus infection of the nasal passages in the case of the bovine might set up secondary inflammatory processes in the surrounding osseous tissue thus creating intra-osseous growth and pressure. This is not unlikely in the bovine where the sinusoidal spaces of the nasal passages very largely surround the eye. Further should this occur early in calfhood, permanent damage would result. It would be impossible to recover, even though vitamin A or carotene were given. Recovery from some of the symptoms was slow or nil in the cases where small remedial doses were tried. These experiments showed plainly that recovery from the developing syndrome was possible only where large overdosages of carotene or vitamin A were given.

The evidence obtained in these experiments indicates clearly that the primary deficiency of this ration is vitamin A in the case of the rabbit. If one or more deficiency factors are the causative agent or agents for the atypical blindness of cattle one must assume that the dietary requirements of the rabbit for the factor are either extremely low or unnecessary, or that the rabbit can synthesize it. Since excellent performance and even the added burden of pregnancy were obtained when ample carotene was administered it seems more logical to assume that the deficiency is an inadequate source of vitamin A. This is all the more likely since little is known concerning the various factors which condition the absorption and conversion of carotene into vitamin A and its utilization. Unpublished data of Phillips and Hart ('36) indicate that certain types of ration distinctly increase the vitamin A requirement of the chick when carotene is the sole source of the vitamin.

SUMMARY AND CONCLUSIONS

Experiments using a diet which caused optic foramen stenosis in calves failed to produce stenosis in the rabbit. The ration did produce a syndrome in the rabbit otherwise strikingly similar to that produced in calves. The effects upon growth, equilibrium, and the eye resembled the syndrome which produced stenosis of the optic foramen in calves. The development of this syndrome could be obviated by feeding adequate quantities of carotene or vitamin A. Fifty micrograms of carotene per kilogram of body weight afforded protection and the maintenance of health. Wesson oil, aerated cod liver oil, or a flavin concentrate were without the preventive or remedial effect. The remedial dose of carotene was distinctly above the preventive level of 50 micrograms per kilogram of body weight. Small doses, 30 to 70 micrograms per kilogram of body weight, were without remedial effect but if the dosage were increased from six to twenty times, recovery took place. One hundred per cent mortality was experienced unless a source of vitamin A was added.

These data indicate that an adequate source of vitamin A was the primary deficiency of this ration and that sub-minimal amounts of vitamin A may produce various physiological reactions which are the results of both primary and secondary tissue responses.

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THE MEASUREMENT OF THE EFFICIENCY OF DIETS. NEW APPARATUS AND PROCEDURES ¹

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FOUR FIGURES

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Within the memories of the middle-aged of today nutrition was understood to be a comparatively simple process, and nutritive values of foods were measured, with general confidence and satisfaction, in terms of calories, digestible nutrients, metabolizable energy or net energy.

During the current century, however, the science of nutrition has been, in effect, born anew—so great has been the advance in our knowledge; and, as each revolutionary discovery has followed the one before, thus contributing to our understanding of the subject as one of vast complexity, the idea of evaluating foods in terms of common measures has been pushed so far into the background as to appear to be ever less and less practicable and significant.

In reality, however, human society has not outgrown the need for measures of food value; we deal with foods in a quantitative way; foods are obtainable in only limited amounts; and nutritive values in relation to cost will remain always a consideration of first-class economic importance.

An especially baffling feature of this whole matter has been the growing appreciation, to which this laboratory has contributed a long series of studies, of the fact that foods cannot be scientifically evaluated, individually, in any common terms, since each essential nutrient, at least finally, if not immediately, affects the utilization of every other.

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It seems to be a fact, however, that nutritive values can be measured, with some degree of consistency as of the more or less completely balanced diets in which foods are used; and it is therefore important that there be generally accepted, standard methods of evaluation of diets, especially as means of expression—in common terms—of the effects of specific nutritive deficiencies.

In this light the present study was undertaken, in a spirit of seeking practical, conventional methods of dietary comparison and evaluation, based, as definitely as practicable, upon scientific understanding and method.

The immediate objective in this investigation was the demonstration of the applicability of the carbon and nitrogen balance method to small animal experimentation, and the comparison of two plans of study of dietary values by this method, with the albino rat as the experimental subject.

EXPERIMENTAL DIETS

Six diets, of significance with reference to human nutrition, were compounded in such manner that each would have approximately the same nitrogen and gross energy values; and these diets were compared, as above stated, by two radically different plans of calorimetric experimentation, depending, in both cases, on the carbon and nitrogen balance procedure, which, in the course of many years' work with cattle, at this institute, has yielded essentially the same values for heat production as has direct calorimetry.

The diets were compounded from the following constituents:

<i>Diet no.</i>	<i>Components</i>
1	Wheat and whole milk powder
2	Bread, butter, beef and roots
3	Bread, butter, beef, and green vegetables
4	Bread, butter, beef and fruits
5	Peanuts, fruits and green vegetables
6	Corn meal, navy beans, pork cracklings and molasses

The percentage composition of the diets is given in table 1. Diet no. 1 was exceedingly simple, but, normally, of nearly maximum nutritive value; nos. 2, 3 and 4 consisted of meat,

TABLE 1
Composition of diets¹; air-dry basis²

Experi- ment no. 1	Diet no. 1	Wheat	Milk powder	NaCl	Protein	Cal. per gm.	Peach	Prune	Orange juice	Tomato	NaCl	Protein	Cal. per gm.
		Peanut	Celery	Green beans	Lettuce	Spinach	Spinach	Prune	Orange juice	Tomato	NaCl	Protein	Cal. per gm.
Experi- ment no. 2	5	30.15	5.45	5.45	5.45	5.45	5.45	9.41	14.12	14.12	0.99	18.44	4634
	1	Wheat	Milk powder	NaCl	Protein	Cal. per gm.							
		47.10	51.91	0.99	17.44	4631							
	2	Beef	Bread	Butter fat	White potato	Sweet potato	Carrot	Beet	NaCl	Protein	Cal. per gm.		
		9.03	50.07	8.22	19.01	6.34	3.17	3.17	0.99	17.13	4601		
	3	Beef	Bread	Butter fat	Celery	Green beans	Lettuce	Spinach	NaCl	Protein	Cal. per gm.		
		5.24	72.93	7.17	3.42	3.42	3.42	3.42	0.99	17.06	4612		
Experi- ment no. 2	4	Beef	Bread	Butter fat	Peach	Prune	Orange juice	Tomato	NaCl	Protein	Cal. per gm.		
		8.67	59.84	7.23	4.65	4.65	6.98	6.98	0.99	17.56	4620		
	5	Peanut	Celery	Green beans	Lettuce	Spinach	Peach	Prune	Orange juice	Tomato	NaCl	Protein	Cal. per gm.
		30.15	5.45	5.45	5.45	5.45	9.41	9.41	14.12	14.12	0.99	18.44	4634
	6	Corn meal	Pork crack- lings	Navy beans	Molasses	NaCl	Protein	Cal. per gm.					
		58.76	22.37	11.35	6.53	0.99	17.69	4695					

¹ All values are per cent except those for calories per gram.

² All products were in the dried (air-dry) condition, except for molasses, which contained its normal percentage of moisture.

bread and butter, as basic constituents, each supplemented with a different type of vegetable food; no. 5 was composed of vegetable products alone, with roasted peanuts as the main source of protein; and no. 6, of mixed animal and vegetable origin, possessed a certain superficial acceptability, but was probably highly inadequate in some fundamental respects.

In this outline of the nature of the diets, the 'roots' were white potato, sweet potato, carrot and beet; the 'green vegetables' were celery, string beans, lettuce and spinach; and the 'fruits' were peach, prune, orange juice and totato—all in the dried (air-dry) form.

The beef preparation was made by drying and pulverizing fresh beef, which had been freed from visible fat and connective tissue. After grinding, in a meat chopper, the fresh meat was heated on a steam bath to a temperature sufficient to accomplish slight cooking, in order to prevent spoilage during drying, after which it was dehydrated at about 65°C. in a hot air oven, and was then finely ground.

PLAN OF EXPERIMENTATION

The general background data relating to experiments nos. 1 and 2 are given in tables nos. 2 and 3, respectively.

The method of the first experiment was to compare two of the diets, nos. 1 and 5, by means of two groups of young rats selected as for paired feeding, in growth and metabolism studies, an unusual condition of this experiment being that exactly the same quantity of food (6.2 gm. per day) was given to each rat, from the beginning to the end of the test.

Three-day periods of metabolic measurement were conducted, by the carbon and nitrogen balance method, at intervals of 2 or in some cases 3, or 4 weeks, there being seven such periods with diet no. 1, and four with diet no. 5.

In the second experiment the six diets described in table no. 1 were compared, each with a different group of six, young, growing, albino rats, selected for uniformity of age and weight; and it was planned that each such group would be subjected to the same series of five metabolic measurements,

by the carbon and nitrogen balance procedure, as indicated below:

1. Fast; preliminary feeding, at the maintenance level, during 6 days, followed, after 1 day, by a fasting heat measurement during 24 hours.

TABLE 2
Experiment 1: Schedule of experimentation

RESPIRATION PERIOD NO.	DIET NO. AND CONSTITUTION	AGE OF RATS ¹	WEIGHT OF RATS	FRESH WEIGHT OF FOOD	DEY WEIGHT OF FOOD	NITROGEN OF FOOD	CARBON OF FOOD	ENERGY OF FOOD
		<i>weeks</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>mg.</i>	<i>gm.</i>	<i>Cal.</i>
1	1. Wheat, milk powder	7	110	6.2	5.747	181	2.770	29.03
2	1. Wheat, milk powder	9	129	6.2	5.747	181	2.770	29.03
3	1. Wheat, milk powder	11	146	6.2	5.747	181	2.770	29.03
4	1. Wheat, milk powder	13	163	6.2	5.747	181	2.770	29.03
5	1. Wheat, milk powder	15	178	6.2	5.747	181	2.770	29.03
6	1. Wheat, milk powder	19	192	6.2	5.747	181	2.770	29.03
7	1. Wheat, milk powder	22	195	6.2	5.747	181	2.770	29.03
1	5. Peanuts, dried fruits, green vegetables	8	96	6.2	5.995	183	2.770	28.73
2	5. Peanuts, dried fruits, green vegetables	10	109	6.2	5.995	183	2.770	28.73
3	5. Peanuts, dried fruits, green vegetables	12	119	6.2	5.995	183	2.770	28.73
4	5. Peanuts, dried fruits, green vegetables	14	129	6.2	5.995	183	2.770	28.73

NOTE: Six rats were used with each treatment in each period, except that with diet no. 2, in periods nos. 3 and 4, only three and two rats, respectively, were used.

¹ At time of respiration measurement.

2. Maintenance; preliminary feeding, at the maintenance level, 4 days; followed by excreta collection during 7 days, and respiration measurement during 3 days.

3. Growth; preliminary feeding, on the production level, 4 days, followed by excreta collection during 7 days, and respiration measurement during 3 days.

4. Maintenance; the time intervals and treatments the same as for the first maintenance period.

5. Fast; heat measurement during 24 hours, beginning 1 day after the termination of the preceding respiration measurement at the maintenance level.

In the conduct of this experiment, however, a departure was made from this schedule, in one important detail, regarding the preliminary feeding for the first period (fast), which will be discussed later.

TABLE 3

Experiment 1: Partition of average energy consumed per rat per day¹

PERIOD	FEED	FECES	URINE	PROTEIN RE- TAINED	FAT RE- TAINED	HEAT PRODUC- TION	FECES AND URINE	BODY GAIN	LIVE WEIGHT
Diet 1: milk powder, wheat									
	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>gm.</i>
1	29.03	2.53	1.23	2.62	2.28	20.37	3.76	4.90	110
2	29.03	2.63	1.34	1.74	3.27	20.06	3.97	5.01	129
3	29.03	2.55	1.48	1.32	2.12	21.57	4.03	3.44	146
4	29.03	2.73	1.42	1.58	1.66	21.64	4.15	3.24	163
5	29.03	2.84	1.45	1.37	0.18	23.19	4.29	1.55	178
6	29.03	2.69	1.56	0.77	-0.22	24.24	4.25	0.55	192
7	29.03	3.06	1.68	0.38	0.20	23.71	4.74	0.58	195
Diet 5: peanuts, fruits, green vegetables									
1	28.73	6.79	1.27	1.10	1.42	18.15	8.06	2.52	96
2	28.73	6.52	1.46	0.94	1.24	18.57	7.98	2.18	109
3	28.73	5.80	1.56	1.16	1.37	18.84	7.36	2.53	119
4	28.73	5.78	1.60	1.05	0.12	20.19	7.38	1.17	129

¹ Each value is an average of results obtained with six rats.

THE CARBON AND NITROGEN BALANCE METHOD

The procedure used for the measurement of the heat production, known as the carbon-and-nitrogen balance method, depends on 1) a measured and constant food intake, 2) the collection and analysis (carbon, nitrogen and energy) of the urine and feces, and 3) the measurement of the CO₂ eliminated by the lungs.

From the gain of nitrogen are computed the equivalent quantities of protein, carbon and energy gained.

From the intake of carbon are subtracted the quantities of carbon eliminated in urine, feces and CO_2 , and the carbon content of the protein gained, the remainder being the carbon gained as fat.

Finally, the heat production is computed by subtracting from the energy intake the energy of the feces and urine, and of the energy gain as protein and as fat.

An illustration of this computation follows:

Daily balance

	DRY MATTER	NITROGEN	CARBON	ENERGY
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>Cal.</i>
Feed		181	2770	29.03
Feces		26	290	3.25
Urine		146	143	1.64
CO_2			2293	
Protein	54	9	28	0.31
Fat	21		16	0.20
Heat production				23.63

The special advantage of the carbon and nitrogen balance method is that it permits the measurement of the total effect of a diet on the metabolism of an animal, including values for protein and fat gained or lost, as well as the heat production, for protracted periods, under normal conditions as to physical activity.

A basic assumption involved—that the carbohydrate content of the body is the same at the end as at the beginning of the balance period—has been found during more than a quarter century of experimental work at this institute, with cattle, in similar, normal states of nutrition, to be fully warranted; and even though there were a change in the carbohydrate content of the body, the resultant errors in the computed heat production and body gain of energy would be slight, since the energy values of the carbon of fat and carbohydrate are closely similar.

DESCRIPTION OF APPARATUS AND ROUTINE OF OPERATION

The apparatus used for determining the carbon and nitrogen balances, shown in figure 1, consisted of a cylindrical, water-sealed chamber, made of tin-coated, sheet copper, inside which the rat was confined in a cage constructed of $\frac{1}{8}$ inch mesh galvanized iron screen.

This cage was 8 inches in diameter, and 10 inches high, with a false bottom of $\frac{1}{2}$ inch mesh galvanized iron screen placed 1 inch above the lower edge of the cage. During use this cage was set into a crystallizing dish, the inside dimensions of which were $8\frac{3}{8}$ inches diameter by $2\frac{3}{4}$ inches height.

The lower edge of the cage was held $1\frac{1}{4}$ inches above the bottom of the crystallizing dish by three wires soldered to the cage in such position as to support it from the edge of the dish; and below the cage a $\frac{1}{8}$ inch mesh stainless steel wire screen, supported from the bottom of the dish, retained the feces, and permitted the urine to fall through to the bottom, from which it was collected.

The feeding cup and holder were constructed as described and illustrated in an earlier paper by Forbes, Swift, Black and Kahlenberg ('35).

During the determination of the metabolizable energy of a diet, prior to the conduct of a respiration experiment, the rat serving as the subject was confined in this cage by an inverted pie tin placed over the top.

During respiration measurements the rat and cage were placed inside the chamber shown in the figure.

This chamber was provided with two windows, 4 inches \times 6 inches in dimensions. The windows were of celluloid, cemented onto brass window frames. These frames were cut from $\frac{1}{16}$ inch sheet brass, and were soldered inside the copper cylinder, the opening in the cylinder being $\frac{1}{2}$ inch larger, each way, than the opening in the brass plate forming the window frame, thus leaving a $\frac{1}{4}$ inch edge of brass plate exposed on each side of the opening. The celluloid window pane was cemented onto this exposed $\frac{1}{4}$ inch edge of brass plate.

The passage of a ventilating air stream through the chamber was provided for by an entrance tube in the side wall at the height of the false bottom in the inside cage, and an exit tube near the top of the side wall.

Food was introduced into the feeding cup, during a respiration experiment, through a copper funnel, soldered into the top of the cylinder. This funnel was made by 'spinning' in order that it would be smooth inside, and could be tightly closed with a rubber stopper.

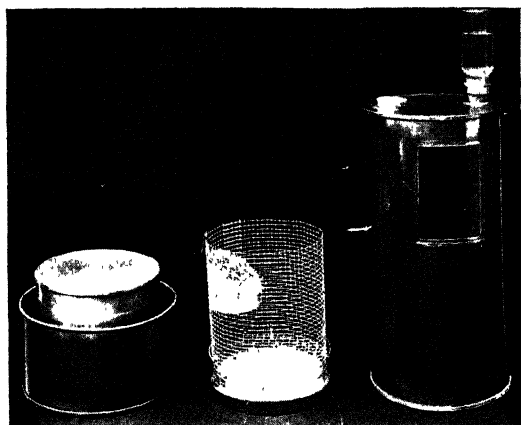


Fig. 1 Respiration chamber, used to determine the carbon and nitrogen balance.

During feeding, the ventilation was stopped, and, before the feeding funnel was opened, the internal pressure was allowed to equalize with the outside pressure, through the soda-lime tube—the internal pressure being slightly less than the outside pressure, by an amount equivalent to a 2-inch column of water.

The animal was watered by the device shown in the illustration. The watering tube, the lower end of which is visible through the window (fig. 1) passes through two rubber stoppers, one of which closes the bottle, and the other is held by a collar of brass tubing soldered into the top of the cylinder.

During a respiration experiment air was drawn through the chamber at the rate of 0.75 liter per minute. The air flow

was regulated to a rate measured by means of a manometer, containing water, with a capillary tube inlet in the suction line. This device was calibrated with the aid of a graph made by establishing a number of points from which a curve was drawn representing rise of water in the suction arm of the manometer corresponding to rate of air flow through the apparatus.

In the establishment of each point on this curve, representing a different rate of air flow, the rate of flow (abscissa) was first measured with a Bohr meter, attached at the intake end of the absorption train; and then, with the Bohr meter replaced by the regulating device, but with the same suction prevailing, the rise of water in the manometer (ordinate) was measured.

During the determination of metabolizable energy, before the cage was placed inside the respiration chamber, the separate collection of urine and feces was made essentially as described in an earlier paper, by Swift, Kahlenberg, Voris and Forbes ('34).

The urine was preserved, during the determination of metabolizable energy, by CuSO_4 and NaF previously introduced into the crystallizing dish in solution, and then evaporated to dryness. Twenty grams NaF and 40 gm. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were made up to 2 L with water, and 50 cc. of this solution was used in each collection dish.

The urine was washed out of the dishes with hot water, at the end of the collection period, and, after being made up to a definite volume, aliquot samples were taken for carbon and nitrogen determination.

The energy of the urine was computed from its carbon content, extensive data from this laboratory having shown that with diets containing at least 15% of protein the energy-carbon ratio is essentially constant at 11.5 Cal. per gram carbon.

During the determination of metabolizable energy the feces were removed daily from the stainless steel wire screen. The feces of each rat were kept separate until the end of the collection period, after which they were air dried and weighed

The feces of the six rats on a given diet were then combined, in order to make a sample sufficiently large for analysis; but the weight of feces from each individual rat was employed in computing the quantities of carbon, nitrogen and energy eliminated therein.

In the experiments to be discussed six of the respiration chambers were used as shown in figure 2, thus providing for the use of six animals, as repeats, on the same diet.

The accessory equipment requires no description except to list the parts, in the order in which the air stream passes through them: H_2SO_4 bottle, soda-lime tube, respiration

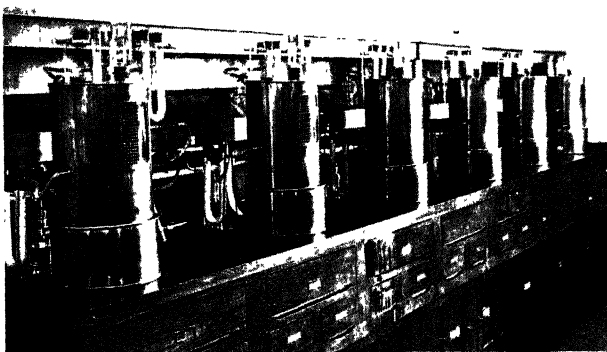


Fig. 2 Respiration chambers equipped as in use.

chamber, H_2SO_4 bottle, acid-pumice check tube, two soda-lime tubes, two acid-pumice tubes, vacuum pump. Also there must be a by-pass for 'sweeping out' the chamber, by providing that the air shall pass directly from the chamber to the vacuum pump.

It was found that during a respiration experiment about 10.4 gm. of water were given off from the water seal into the air stream, but that no CO_2 passed through the water seal.

During feeding, as distinguished from fasting periods, urine and feces were collected only while the cage was outside the respiration chamber, since, in the chamber, the feces remained moist, and underwent offensive decomposition—when handled

in the usual way. During CO_2 measurement, therefore, the stainless steel screen was removed from the crystallizing dish, and the excreta were allowed to drop into a solution of CuSO_4 and NaF , covered by mineral oil, in the crystallizing dish.

During fasting periods the respiration chambers were 'swept out,' through by-passes, for $1\frac{1}{2}$ hours before the beginning of the 24-hour CO_2 measurements. The fasting urine, therefore, was collected during $25\frac{1}{2}$ hours; and, after the urines from the six rats on a single treatment had been combined and analyzed, the results were computed to the basis of one rat for 24 hours.

Since the average 24-hour urinary outgo of carbon, nitrogen and energy represented only a total of 6 rat days, however, these values were employed in only a tentative manner in the computation of the heat production.

For comparison with the values thus obtained the heat production was also computed from the CO_2 production, by the use of an assumed respiratory quotient of 0.74, since it had been found, by Kriss, Forbes and Miller ('34), that this R.Q. prevails under such conditions as existed.

The heat production as computed by the second procedure was found in all cases to be lower than as computed by the first method, the average difference being 3.7% of the smaller quantity. The values computed by the second procedure were considered as probably more nearly correct, and these values alone are reported, and utilized in computations.

In the conduct of an experiment, the collection of CO_2 in the first respiration chamber of the series of six was started at 10.00 A.M., and, thereafter, in the remaining chambers at intervals of 15 minutes; and since the first chamber was swept out for $1\frac{1}{2}$ hours, the other chambers were swept out for increasing periods of time reaching $2\frac{3}{4}$ hours for chamber no. 6.

At the end of each 24-hour period the ventilation of each respiration chamber was stopped, for about 10 minutes, to permit the weighing of the absorption tubes. The CO_2 which accumulated during this time was then accounted for in the course of the next experimental day.

DISCUSSION OF RESULTS

Experiment no. 1

This may be regarded as a growth experiment, with results expressed in the form of the familiar growth curve, supplemented by a series of carbon and nitrogen balances, which revealed the progressive changes in the partition of the food energy, and in the character of the growth.

The method of feeding, however, differed, as already stated, from the *ad libitum* procedure commonly followed in growth experiments, in that the same amount of feed was given throughout the test.

This procedure afforded a uniquely searching basis for comparison of the values of the diets, since, as the animal increased in size, it was subjected to an increasingly rigorous test of its ability to maintain itself and to grow on a food supply, which, under the circumstances, must have been progressively less and less adequate to the animal's needs.

The results of experiment no. 1 comprise table 3, and are graphically represented in figures 3 and 4, which exhibit a marked contrast between the values of the two diets used, nos. 1 and 5.

With diet no. 1 the intervals between the seven balance periods were 2, 2, 2, 2, 4, and 3 weeks, respectively, while with diet no. 5 the intervals between the four periods were each 2 weeks. The placing of the balance periods, in point of time, is indicated in the graphs by the separate, short lines, apart from the growth curves.

The partition of the energy of the diets shows that the most prominent difference between their nutritive values was in the digestibility of the energy producing nutrients—as indicated by the caloric equivalent of the feces.

The superiority of diet no. 1 to no. 5 was also indicated by the smaller amounts of energy in the urine, the larger amounts retained as fat and as protein, and the larger gains in live weight.

The heat production from diet no. 1 exceeded that from diet no. 5, but the proportion of the metabolizable energy eliminated as heat from diet no. 5 exceeded that from diet no. 1.

At the termination of period 4 the rats on diet no. 5 were killed. The hair was rough, and the general appearance unhealthy. The feces were small, numerous and black. The

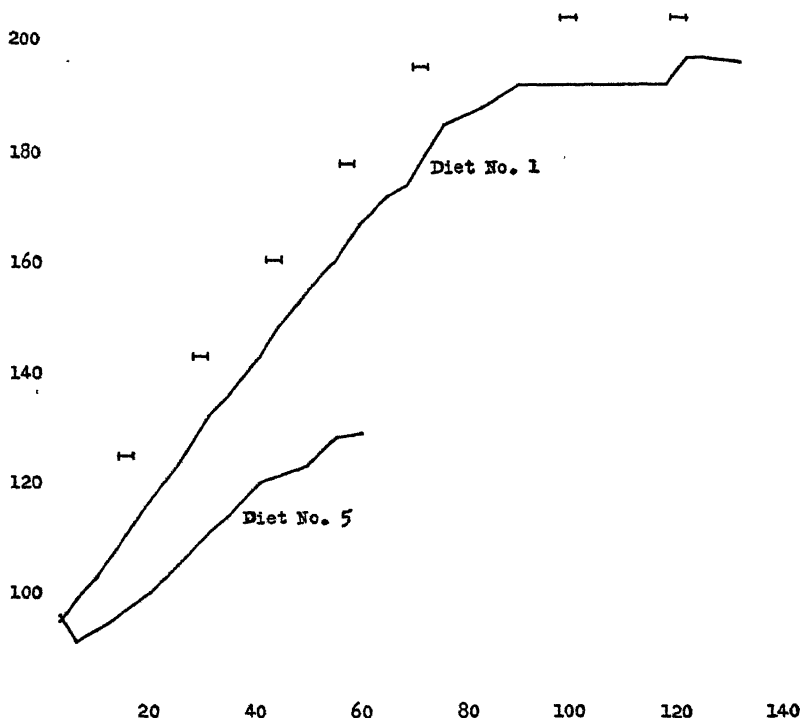


Fig. 3 Curves representing the average growth of six rats, with a constant rate of food intake, on each of two diets.

eyes were light colored; the caeca were distended, and nodules were present on the small intestines.

Of especial interest are the changes which took place in the disposal of the constant food energy as the experiments progressed. Thus, the daily energy gain, in the case of diet no. 1, decreased from 4.90 Cal. per day, in period 1, to 0.58 Cal.

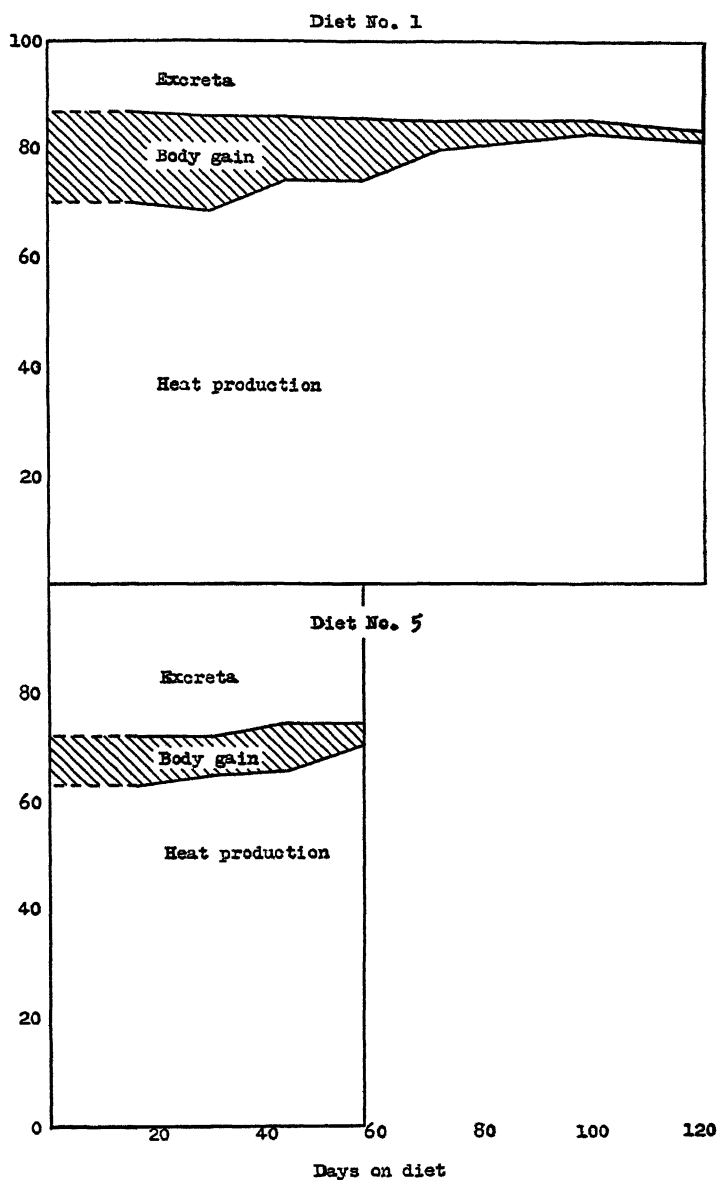


Fig. 4 Representing the average partition of the food energy of six rats, with a constant rate of food intake, on each of two diets.

per day, in period 7. This decrease in gain is necessarily associated with a like increase in the energy leaving the body in the excreta and as heat, about 23% of the decrease in gain being accounted for by an increase in energy of excreta, and 77% by increased heat production.

At the end of the experiment the food intake was sufficient but little more than to keep the animal in protein and energy equilibrium.

It is also important to note that the heat production at the beginning of the experiment was relatively much greater than at the end, in consideration of the fact that the live weight increased from 110 to 195 gm.

The regularity in the changes which took place in the disposal of feed energy throughout the experiment, as shown in table 1, is also notable.

After the termination of the fourth balance period with the rats on both diets, those on diet no. 1 were continued in order to follow through the changes in food disposal as the animals made further growth. Thus, a decrease took place in body gain from 1.55 Cal. to 0.55 Cal. over a period of 4 weeks.

The rats which received diet no. 5 did not consume their feed satisfactorily after the first two periods; and in periods 3 and 4, only three and two rats, respectively, conformed to the experimental routine. It was characteristic of the diet, therefore, that, in the course of time, it came not to be well accepted.

The total energy gained by each group of rats during the first 8 weeks may be computed by assuming a constant rate of change between the bi-weekly points of observation. On such a basis the total gain per rat of those which received diet no. 1 during the first four periods (8 weeks) was 243 Cal. For those on diet no. 5 the gain was 126 Cal.

Total gains might also be determined, at the end of such a series of observations, by analysis of the rat bodies.

A study of the results from 168 rat days of this experiment shows that the CO_2 measurements under the prevailing conditions, which allowed the animal the freedom of the cage, were satisfactorily concordant.

Among fifty-four values for daily CO_2 outgo, computed from 3-day respiration measurements, and three such values for 2 days (no data being excluded), the average deviation was 1.1% of the mean, the greatest deviation being 2.8%.

In a preliminary test period with six rats of 100 gm. average live weight it was found that 5.0 gm. daily of diet no. 1 was sufficient exactly to maintain their live weight during 14 days.

Assuming this maintenance requirement to vary as the 0.73 power of the live weight, we may compute that the 6.2 gm. fed in this experiment would permit growth up to a weight of 134 gm., at which time the rat would be forced to use all the feed for maintenance, without growth. However, the intake of 6.2 gm. actually allowed growth up to a live weight of 195 gm., which evidence of increasing efficiency of utilization of food energy may be considered as at least in part due to diminishing use of food energy for maintenance. Maintenance requirements of energy, therefore, should be determined with the subjects as nearly as possible in energy equilibrium. The profound effect of undernourishment on basal metabolism (per unit of surface area) has long been known (Zuntz, '13; Magnus-Levy, '06; Chittenden, '04).

In this comparison of the two diets of equal protein and energy contents, one composed of milk powder and wheat, and the other of roasted peanuts, dried fruits and dried vegetables, the first diet mentioned sustained the prevailing understanding that it is highly efficient, while the second diet mentioned, in spite of a superficial equivalence to the diet of wheat and milk powder, was convincingly shown to be highly deficient.

The further discussion of the results of this experiment will be found following the description of the second experiment.

Experiment no. 2

The second program of experiments in this investigation is outlined in table 4, the general plan and objectives having been already explained.

When this program was outlined it was intended that the values for heat production from the two fasting periods would be averaged, to serve as a base value in computing the heat increments and net energy values of the diets for maintenance; and, similarly, that the results obtained in the two maintenance periods would serve as a basis for computing the heat increments and net energy values of the diets for growth.

TABLE 4

Experiment 2: Schedule of experimentation, representing average values per rat per day¹

NUMBERS AND CHARACTER OF DIETS	PERIOD NOS.	PLANE OF NUTRITION	AGE OF RATS	WEIGHT OF RATS ²	FRESH WEIGHT OF FOOD	DRY WEIGHT OF FOOD	NITROGEN OF FOOD	CARBON OF FOOD	ENERGY OF FOOD
			<i>weeks</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>mg.</i>	<i>gm.</i>	<i>Cal.</i>
1									
Wheat, milk powder	3	Growth	10	129	7	6.441	195	3.079	32.42
	4	Maintenance	12	133	5	4.601	140	2.200	23.16
	5	Fast	12	134	0	0	0	0	0
2									
Bread, butter, beef, roots	3	Growth	10	143	7	6.661	192	3.123	32.21
	4	Maintenance	12	149	5	4.758	137	2.231	23.01
	5	Fast	12	151	0	0	0	0	0
3									
Bread, butter, beef, green vegetables	3	Growth	10	140	7	6.703	191	3.063	32.28
	4	Maintenance	12	146	5	4.788	137	2.188	23.06
	5	Fast	12	147	0	0	0	0	0
4									
Bread, butter, beef, fruits	3	Growth	10	142	7	6.722	197	3.123	32.34
	4	Maintenance	12	145	5	4.802	141	2.231	23.10
	5	Fast	12	148	0	0	0	0	0
5									
Peanuts, fruits, green vegetables	3	Growth	10	116	7	6.769	207	3.128	32.44
	4	Maintenance	12	119	5	4.835	148	2.234	23.17
	5	Fast	12	120	0	0	0	0	0
6									
Corn meal, beans, cracklings, molasses	3	Growth	10	136	7	6.403	198	3.071	32.87
	4	Maintenance	12	139	5	4.574	142	2.194	23.48
	5	Fast	12	139	0	0	0	0	0

¹ All values are averages of results obtained with six animals.

² In fasting periods the live weights were taken 24 hours after the last feed was given; in other periods the weights are averages of weights taken at the beginning and end of respiration periods.

After the feeding and metabolism tests were finished, however, it was found that the plan of experimentation had miscarried in one important particular—that the rats had not been subjected to the 6-day feeding at the maintenance plane of nutrition, preliminary to the first determination of the heat production of fast, but that this fast had followed feeding at a rate so much in excess of maintenance that the fasting heat production generally exceeded that of the following maintenance period, the heat increments being negative quantities, with four rats among the six.

The first fasting heat measurements (period 1) for each group of treatments, therefore, were discarded as invalid, and the heat increments of maintenance were based on the second fasting heat measurements (period 5) alone. This incident serves the important purpose of emphasizing the significance of the plane of nutrition preceding the measurement of fasting katabolism, as affecting the magnitude of such measurements.

In recognition of the fact that the habit of an animal, with reference to heat production, as determined by the plane of nutrition to which it is accustomed, prominently affects metabolism during a subsequent fasting period, Forbes, Braman and Kriss ('30) recommended that feeding on the plane of energy equilibrium be followed as a standard treatment preliminary to the determination of the heat production of fast.

Having discarded the results of period 1, with each diet, therefore, there remained the question as to whether to base the computation of the heat increment of the food for growth (period 3) on an average of the results obtained in the two maintenance periods (periods 2 and 4), or on the second (period 4) alone.

While these two values were, presumably, equally valid, it was decided to use the second (period 4) alone, since the live weights in periods 3, 4 and 5 differed but little, thus requiring only small corrections of the heat production in computing to correspond to an average live weight, while a much

larger correction would have been necessary in the results from period 2, the first maintenance period, if these had been used.

On this account the results of experiment 2 depend on periods 3, 4 and 5 alone. Accordingly it is suggested that, in the use of the procedure described, for the evaluation of diets, the experimental subjects need be given only the three treatments—growth, maintenance, and fast—in the order named, which provides the proper preliminary treatment for the fasting heat measurement.

The average daily heat production per rat is given in table 5, computed to the basis of the average empty live weight, that is, the weight of the animal minus the contents of the alimentary tract.

These empty live weights were determined, for each diet, by means of a specially selected, representative group of rats, which were subjected to a feeding experiment for this purpose alone.

The correction of the heat production was made, in each case, in accord with the 0.73 power of the live weight, with the heat production of fast as the basis of correction.

The main results of the experiment are given in table 6, the upper half of the table representing the utilization of the diets for maintenance (period 4), and the lower half for growth (period 3).

The metabolizable energy which was computed as the gross energy minus the energy of the feces and urine, with a correction for the non-metabolizable fraction of the body gain, was unaffected by the plane of nutrition; but, with the two groups (maintenance and growth), each diet was characterized by an individual metabolizability.

In this relation the diets of special interest are no. 6 (corn meal, beans, cracklings, molasses), because of its maximum metabolizable energy value, no. 5 (peanuts, fruits, green vegetables), because of its minimum value, and no. 4 (bread, butter, beef, fruits) because it had a distinctly lower metabolizability than diets nos. 2 and 3, which differed from no. 4 only in the character of the vegetable component.

As compared with roots and green vegetables, fruits exercised a slight influence to lower the metabolizability of the diet in which it was contained.

The maximum metabolizability of diet no. 6 may be considered to be due especially to the high digestibility of the

TABLE 5
Experiment 2: Average daily heat production per rat

NUMBER AND CHARACTER OF DIETS	PERIOD NO.	PLANE OF NUTRITION	EMPTY LIVE WEIGHT	HEAT PRODUCTION	FASTING KATABOLISM ¹	CORRECTED HEAT PRODUCTION ¹
			<i>gm.</i>	<i>Cal.</i>	<i>Cal.</i>	<i>Cal.</i>
1 Wheat, milk powder	3	Growth	122	22.30		23.36
	4	Maintenance	129	19.14		19.46
	5	Fast	130	18.78	18.99	18.99
2 Bread, butter, beef, roots	3	Growth	138	22.98		22.43
	4	Maintenance	146	20.87		19.60
	5	Fast	148	18.09	16.64	16.64
3 Bread, butter, beef, green vegetables	3	Growth	136	23.18		22.80
	4	Maintenance	142	21.97		21.02
	5	Fast	143	18.48	17.43	17.43
4 Bread, butter, beef, fruits	3	Growth	137	22.02		21.58
	4	Maintenance	139	19.97		19.36
	5	Fast	142	16.80	15.93	15.93
5 Peanuts, fruits green vegetables	3	Growth	102	19.04		21.86
	4	Maintenance	111	16.86		18.82
	5	Fast	112	14.59	16.45	16.45
6 Corn, meal, beans, cracklings, molasses	3	Growth	132	23.82		23.82
	4	Maintenance	136	22.43		22.04
	5	Fast	136	18.27	17.88	17.88

¹ Corrected to represent the average empty live weight of 132 gm.

sugar of the molasses, and to the fat of the corn meal and cracklings; and the minimum metabolizability of diet no. 5, to the fact that it contained no highly digestible component.

There was a difference of 27.3% between the metabolizable energy values of diets nos. 5 and 6, based on the value of no. 5.

TABLE 6

Experiment 2: Metabolizable energy, heat increments and net energy of diets for maintenance and for growth

NUMBERS AND CHARACTER OF DIETS	PLANE OF NUTRITION	FOOD, DRY MATTER	METABOLIZABLE ENERGY PER GRAM D. M.	HEAT PRODUCTION ¹	HEAT INCREMENT PER GRAM D. M.	NET ENERGY PER GRAM DRY MATTER
		gm.	Cal.	Cal.	Cal.	Cal.
1 Wheat, milk powder	Maintenance	4.601	4.30	19.46	0.10	4.20
2 Bread, butter, beef, roots	Maintenance	4.758	4.25	19.60	0.62	3.63
3 Bread, butter, beef, green vegetables	Maintenance	4.788	4.29	21.02	0.75	3.54
4 Bread, butter, beef, fruits	Maintenance	4.802	4.12	19.36	0.71	3.41
5 Peanuts, fruits, green vegetables	Maintenance	4.835	3.62	18.82	0.49	3.13
6 Corn meal, beans, cracklings, molasses	Maintenance	4.574	4.52	22.04	0.91	3.61
1 Wheat, milk powder	Growth	6.441	4.30	23.36	2.12	2.18
2 Bread, butter, beef, roots	Growth	6.661	4.24	22.43	1.49	2.75
3 Bread, butter, beef, green vegetables	Growth	6.703	4.31	22.80	0.93	3.38
4 Bread, butter, beef, fruits	Growth	6.722	4.15	21.58	1.16	2.99
5 Peanuts, fruits, green vegetables	Growth	6.769	3.55	21.86	1.57	1.98
6 Corn meal, beans, cracklings, molasses	Growth	6.403	4.52	23.82	0.97	3.55

¹ Computed to the basis of the average empty live weight of 132 gm.

The values for heat production corresponded, in a general way, to the metabolizable energy, and seem not to be especially noteworthy.

The energy balances during feeding on the maintenance plane varied between -1.57 and $+0.82$ Cal., and the values for heat production, as computed to the basis of the average empty live weight of the rats, were reasonably concordant. The heat increments for maintenance, therefore, rest on a fairly satisfactory basis.

The heat increment values of the diets for growth are in certain respects noteworthy. Thus, the increment of diet no. 1 (wheat, milk powder) is much higher than that of any other; and the increments of diets nos. 3 (bread, butter, beef, green vegetables) and no. 6 (corn meal, beans, cracklings, molasses) are the lowest of all.

Corresponding to the lowest heat increment for maintenance (diet no. 1; wheat, milk powder) is the highest net energy for maintenance. The net energy, for maintenance, of diet no. 5 (peanuts, fruits, green vegetables) was distinctly low; while diets nos. 2, 3, 4 and 6 had nearly the same net energy for maintenance.

The net energy value for each diet was consistently lower for growth than for maintenance.

Leaving out diets nos. 6 and 2, the net energy of the diets for growth, and the nitrogen retention, and the ratio of protein to non-protein energy stored, were all in the same order, that is, in order of ascending values—diets nos. 5, 1, 4, 3.

The fact that diet no. 6 was characterized by the maximum net energy value for growth appears to have been due to the same circumstances that gave to this diet its maximum metabolizable energy value, that is, the high sugar content of the molasses, and the relatively high fat contents of the corn meal and the cracklings; and, similarly, the fact that diet no. 5 was characterized by the minimum net energy value for growth seems to have been due to the same fact that gave this diet its minimum metabolizable energy value, that it contained no highly digestible component.

Aside from these two values the most notable is the low net energy of diet no. 1 (wheat, milk powder) for growth. This is most readily understandable on the assumption (for which, however, there is no warrant except hypothesis) that the protein value was injured during the drying of this particular lot of milk.

It is necessary not to be misled by high net energy values for body increase of diets which, in view of their composition, would be considered poor foods for growth, since such diets may not be efficient for the production of protein increase. In other words, net energy is not a measure of protein value.

The digestibility of the experimental diets appears not to have been affected by the plane of nutrition in any generally significant manner. The outstanding facts observable in the data, as presented in table 7, are that diet no. 5, of peanuts, fruits and green vegetables, was much lower in digestibility of both energy and nitrogen, at both planes of nutrition, than was any other; that the digestibility of the energy of diet no. 3, of bread, butter, beef and green vegetables, was higher than that of any other, at both planes of nutrition; and that the digestibility of the nitrogen of diet no. 1, of wheat and milk powder, and no. 3, of bread, butter, beef and green vegetables, was appreciably higher, at both planes of nutrition, than was that of any others.

In consideration of gross efficiency—based upon total intake rather than intake in excess of the maintenance requirement—the quantities of nitrogen and energy retained by the animals on diets approximating the maintenance level show that this group of diets were more favorable to nitrogen than to energy retention. Diet no. 1, of wheat and milk powder, however, was lowest in nitrogen storage at the lower plane, and next to the lowest at the higher plane, which suggests again that the protein value of the particular lot of milk powder used in experiment no. 2 had been damaged during drying.

At the higher plane of intake there was 70% difference between the largest and the smallest quantities of nitrogen stored from these diets (nos. 5 and 2, respectively); also the

TABLE 7
Experiment 2: Average digestibility and daily retention of energy and nitrogen of diets

DIET NO.	CHARACTER OF DIET	PLANE OF FOOD INTAKE	ENERGY			NITROGEN			RATIO OF PROTEIN TO NON-PROTEIN ENERGY RETAINED
			Food	Digested	Retained	Food	Digested	Retained	
			<i>Cal.</i>	%	<i>Cal.</i>	<i>mg.</i>	%	<i>mg.</i>	
1	Wheat, milk powder	Maintenance	23.16	91.8	0.79	140	89.3	17	
2	Bread, butter, beef, roots	Maintenance	23.01	93.2	—0.38	137	84.7	33	
3	Bread, butter, beef, green vegetables	Maintenance	23.06	94.3	—1.15	137	89.1	35	
4	Bread, butter, beef, fruits	Maintenance	23.10	91.0	0	141	82.3	25	
5	Peanuts, fruits, green vegetables	Maintenance	23.17	82.3	0.82	148	72.3	23	
6	Corn meal, beans, cracklings, molasses	Maintenance	23.48	92.8	—1.57	142	83.8	26	
1	Wheat, milk powder	Growth	32.42	91.7	5.82	195	89.2	60	0.54
2	Bread, butter, beef, roots	Growth	32.21	92.7	5.87	192	82.8	80	0.88
3	Bread, butter, beef, green vegetables	Growth	32.28	94.3	6.28	191	88.5	77	0.72
4	Bread, butter, beef, fruits	Growth	32.34	91.7	6.43	197	84.8	71	0.61
5	Peanuts, fruits, green vegetables	Growth	32.44	80.4	5.36	207	72.0	47	0.43
6	Corn meal, beans, cracklings, molasses	Growth	32.87	92.8	5.58	198	83.3	65	0.66

nitrogen retention from diet no. 6 of corn meal, beans, cracklings and molasses, was surprisingly efficient.

Among the three bread, butter and beef diets (nos. 2, 3 and 4), the proportion of protein to non-protein energy stored was favored first by the roots, second by the green vegetables, and third by the fruits.

The energy retention at the lower plane of nutrition was at or near zero, as intended, while at the higher plane the storage of energy did not differ greatly in accord with the nature of the diets. At the higher plane, the diet which caused the largest energy storage (no. 4) expressed a 20% greater gross energy efficiency than did the diet (no. 5) from which the smallest quantity of energy was stored.

The ratios of protein to non-protein energy retained suggest, as did the net energy values, that in diet no. 1 (wheat and milk powder) the milk protein was 'below par'; though the protein value was lowest of all in diet no. 5, of peanuts, fruits and green vegetables.

The wheat used in diet no. 1, in both experiments, was of the same lot, but the milk powder in the two experiments differed as to trade name. The question as to the cause of the difference in the nutritive value of diet no. 1, as made with the two lots of milk powder, and as used in the two experiments, recalls the findings of Mitchell and Fairbanks ('35) that the cystine content of milk may be diminished by excessive heat, in drying on a drum; of Sharpenak and Eremin ('35) that the 'total albumin' of milk contains more than three times as much methionine as cystine; and of Rose ('37) that methionine is an essential amino acid, while cystine is not essential.

DISCUSSION

In the first experiment, the demonstrated method of comparison of nutritive values of diets, by means of a series of carbon and nitrogen balances determined in the course of a growth experiment with constant food intake, constitutes a highly critical and significant basis for the comparison of the efficiency of diets for purposes of growth.

This method is noteworthy on account of the facts that it demonstrates the developing effects of dietary differences and deficiencies through comparatively long periods of time, the constant level of food intake leading to increasingly severe nutritive conditions; and also that it avoids certain incompletely justified assumptions involved in net energy determinations.

The results obtained by this method lend themselves especially to expression as 1) the metabolizable energy and the heat production in relation to the food energy. 2) The gain in weight before the limit of such increase is reached. 3) The gains of energy and of protein in relation to food energy and protein.

This procedure, therefore, is well adapted to the comparison of diets; but, because of the fact that it expresses the gross efficiency, instead of the net efficiency point of view—there being no separation of nutriment used for maintenance from nutriment used for gain, it is not adapted to the measurement of nutritive values in terms of units.

The second experiment, conducted in harmony with the net energy conception, entails all of the advantages, and the embarrassments, of this superficially logical, but fundamentally imperfect point of view.

The net energy method leads to measurements in terms of calories, of the values of foods as sources of energy; but this is by virtue of a comparison of results obtained from the feeding of two rations—a basal ration, and another which is the same plus a known quantity of the foodstuff to be evaluated; and by virtue of an assumption that the energy expenses and losses of utilization of the smaller or basal ration are the same when this ration is fed as a part of the supplemented ration as when it is fed alone.

This assumption is superficially logical, but it is not exactly physiological.

Also, the net energy method assumes that the energy expense of food utilization is proportional to the quantity of food eaten, which is not exactly true.

Further, net energy values are lacking in perfection, as practical constants, by virtue of the fact that they are prominently affected by differences in environmental conditions which are frequently encountered.

It is true, however, that despite these imperfections, the method of measurement of food values in terms of net energy has much in its favor, especially for limited purposes of close discrimination, under rigorously controlled conditions of experimentation.

While a basis does not exist for a close comparison of the two methods demonstrated, for the study of the nutritive values of foods, the results are in general harmony, insofar as they are comparable.

SUMMARY

New equipment and two experimental procedures were demonstrated, with growing albino rats as subjects, for the comparison and the measurement of the nutritive values of human diets.

The equipment was a series of water-sealed respiration chambers, for the determination of the quantitative and the qualitative character of metabolism, by means of the carbon and nitrogen balance method, under normal conditions of physical activity of the subjects.

One of the procedures consisted of growth experiments, in the course of which were determined series of carbon and nitrogen balances, at intervals, with the same food intake throughout, for the purpose of observing the character of the metabolism as affected by the progressively increasing severity of the nutritive conditions imposed by the gain in weight of the subjects.

This procedure yielded results in terms of gross efficiency, covering requirements for maintenance and production together.

The second procedure consisted of nutritive measurements, also by means of carbon and nitrogen balances, representing the net energy point of view. Balances were determined

during 1) fast, 2) feeding at the maintenance level, and 3) feeding at a higher plane; and upon these balances were based values for heat increment and net energy, separately for maintenance and for growth.

The two distinctly different methods of experimental management afforded equally distinct means of comparison and measurement of the values of diets as sources of nutritive energy and protein. The respective advantages and limitations of the two procedures are pointed out.

In the comparison of the nutritive values of diets the results obtained by the two procedures were in harmony, insofar as they were comparable.

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FURTHER STUDIES ON THE UNSATURATED FATTY ACIDS ESSENTIAL IN NUTRITION¹

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SIX FIGURES

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The earlier work by Evans and Burr ('27 a, b and '28), by Burr and Burr ('29 and '30), and by Evans and Lepkovsky ('32 a) has clearly established the fact that the rat does not thrive on diets rigidly devoid of fat, but develops a characteristic deficiency disease. The most important 'fat deficiency' symptoms are: a retardation and gradually a complete cessation of growth, scaly condition of skin, renal lesions often manifesting themselves in the appearance of blood in the urine, abnormally high water consumption, and irregularities of ovulation. Gestation, lactation, and male reproductive functions are also considerably impaired (Evans, Lepkovsky and Murphy, '34 a, b). The need of the animal body is not, however, as the above mentioned investigators have demonstrated, for fat in general, but for certain unsaturated fatty acids. The ineffectiveness of saturated fatty acids was shown in a striking manner by Evans and Lepkovsky ('32 b), who fed several preparations of these acids as the sole source of energy (about 60% of the diet) and found that rats on such diets developed a condition similar to the 'fat deficiency.'

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There are also considerable differences between the unsaturated fatty acids in their capacity to cure the 'fat deficiency' syndrome. Only two acids, namely linoleic (Burr and Burr, '29) and linolenic (Burr, Burr and Miller, '32) have so far been found effective, whereas oleic (Evans and Lepkovsky, '32a) and alpha-eleostearic (Burr, Burr and Miller, '32) acids have proved ineffective. Arachidonic acid was also tested by Burr, Burr and Miller, but the result did not seem quite convincing. It was found that an addition of methyl arachidonate as 10% of a mixture of equal parts of the methyl esters of linoleic and linolenic acids slightly decreased the curative potency of the original mixture.

In the beginning of this work the above mentioned fatty acids were the only ones tested. It seemed highly desirable to gather some more information in this field by testing as many new fatty acids as possible and, so doing, perhaps to reach gradually some general conclusions as to why certain fatty acids are capable of curing the 'fat deficiency' syndrome while others are not. During the work reported here the following fatty acids not before tested have been examined with regard to their effectiveness in curing the 'fat deficiency:' erucic, $\Delta^{12:13}$ -oleic, ricinoleic and chaulmoogric. In addition, arachidonic acid was retested, and the effect of different levels of linoleic acid was measured somewhat more accurately than earlier. Also the alcohol corresponding to linoleic acid, linoleyl alcohol, was tested.

EXPERIMENTAL

Preparation of the materials tested. The fatty acids were fed in the form of the methyl or ethyl ester. As Lepkovsky, Ouer and Evans ('35) have shown, these esters are utilized by the rat even at comparatively high levels with almost the same ease as the glycerides. In the course of this work the following substances were tested: methyl linoleate, methyl arachidonate, ethyl eruceate, ethyl $\Delta^{12:13}$ -oleate, methyl ricinoleate, ethyl chaulmoograte and linoleyl alcohol.

Methyl linoleate was prepared from corn oil according to the bromination method of Rollet ('09). The resulting tetrabromostearic acid was recrystallized several times from high-boiling petroleum ether, until it was snow-white and melted sharply at 114.5 to 115°. The debromination was carried out exactly according to the original method, and the methyl ester was distilled under highly reduced pressure (1 to 2 mm.). The final product had an iodine number of 168 and a mean molecular weight (by saponification) of 291 (theory 172.5 and 294, respectively).

Methyl arachidonate was a gift from Prof. J. B. Brown, of the Ohio State University, and Mr. George Y. Shinowara associated with him. According to them it had an iodine number 315 (Wijs 2 hours) and a mean molecular weight 314.8 (theory 319 and 318, respectively).

Erucic acid was isolated from rape seed oil according to Noller and Talbot ('30). The acid (m.p. 33° to 34°) was esterified with absolute ethyl alcohol in the presence of concentrated sulfuric acid, and the ester was distilled in high vacuum. The final product, ethyl eruceate, had an iodine number of 68.5 and a mean molecular weight of 364 (theory 69.3 and 366, respectively).

Ricinoleic acid was prepared from castor oil principally by the method of Rider ('31). The acid was converted into the methyl ester by refluxing it with methanolic hydrochloric acid, and the ester was distilled in high vacuum. The resulting methyl ricinoleate had an iodine number of 78.9 and a mean molecular weight 310 (theory 81.3 and 312, respectively).

The ethyl ester of $\Delta^{12:13}$ -oleic acid may be prepared, as Grün and Czerny ('26) have shown, from the corresponding ester of 12-hydroxystearic acid. If the latter is heated to 220° with 2% of beta-naphthalene sulfonic acid, water is split off and a double bond is formed in the position 12:13. This reaction runs very smoothly and is almost quantitative. Methyl acetylricinoleate was the starting material for the preparation of the ethyl 12-hydroxystearate; it had been prepared in the course of the isolation of ricinoleic acid from

castor oil. This substance was first hydrogenated at 100° and atmospheric pressure with Raney nickel as a catalyst. The Raney metal was activated according to Covert and Adkins ('32). The resulting methyl acetoxystearate was then saponified and reesterified with ethanolic hydrochloric acid. This gave us ethyl 12-hydroxystearate, which after two recrystallizations from acetone (m.p. was then 51.5 to 52°) was treated with beta-naphthalene sulfonic acid according to the method of Grün and Czerny. The resulting ethyl $\Delta^{12:13}$ -oleate is, as these authors have demonstrated, a mixture of about one-third of cis-form and two-thirds of trans-form. A part of this mixture was used as such for the feeding experiments, but in view of the fact that as far as known all naturally occurring fatty acids have the cis-configuration, it was felt desirable also to test the biological activity of the isolated cis-form. Therefore the ester was saponified, and the two isomeric forms were separated by a method described in the work of Grün and Czerny. For our purpose it was not, however, necessary to carry the purification quite so far as these workers did; so the isolated cis-acid contained still some of the trans-form and melted at 11 to 13° (instead of 10°). For feeding tests this acid was esterified with absolute ethyl alcohol and concentrated sulfuric acid. The characteristics of our preparations of ethyl $\Delta^{12:13}$ -oleate were: cis-trans mixture: iodine number 81.7, mean molecular weight 309; cis-form: iodine number 80.9, mean molecular weight 305 (theory 81.8 and 310, respectively).

Ethyl chaulmoograte was kindly furnished by Prof. C. D. Leake of the Medical School of the University of California. The sample was not large enough to permit any chemical tests.

Linoleyl alcohol, a compound which evidently has never before been synthesized, was prepared from methyl linoleate by the reduction method of Bouveault and Blanc ('03). The instructions given by Reid and collaborators ('35) for the preparation of oleyl alcohol were followed in their main points. The yield was good and the product was apparently of high degree of purity. A more complete account of the

preparation and properties of linoleyl alcohol has been given elsewhere (Turpeinen, '38).

Preparation of animals. The methods used were principally the same as those employed earlier in this laboratory by Evans and Lepkovsky ('32 a). Female rats were used throughout. They were weaned on the twenty-first day of life and placed on the following fat-low diet:

Diet 667

Casein (gasoline-washed)	24
Sucrose	72
Salts (McCollum 185)	4

This diet was supplemented with 1) 1.0 gm. of ether-extracted brewer's yeast six times weekly, 2) the non-saponifiable fraction of cod liver oil equivalent to 80 mg. of the original oil six times weekly, and 3) the non-saponifiable fraction of wheat germ oil equivalent to 500 mg. weekly. The positive controls received in addition 60 mg. of an ethyl linoleate preparation (approximately 80% pure) six times weekly.

The non-saponifiable fractions of C.L.O. and W.G.O. were prepared by refluxing the oils with a slight excess of 20% methanolic potassium hydroxide under hydrogen for 1 hour, by diluting the mixture with about twice its volume of distilled water, and by taking the non-saponifiable matter into peroxide-free ether.

The animals were kept three in a cage on wire screens. They were weighed at 5-day intervals, and the incidence of estrus was observed by making vaginal smears daily. The growth of the 'fat-deficient' animals (eighty-two rats) as well as that of positive controls (eight rats) is shown in figure 1. The animals on the fat-low diet plateaued in this case at about 160 gm. The positive controls grew much better, but even they did not quite equal in growth the animals on our stock diets. This was probably due to the inadequacy of the ethyl linoleate dose, for the experiments which will be presented below have demonstrated that about 100 mg. of methyl linoleate daily is needed to produce a maximal renewed growth in 'fat-deficient' female rats. The ovulation of the

'fat-deficient' animals was considerably disturbed, although in this respect there was a great deal of variation among individuals. While a few animals had a normal estrous rhythm,

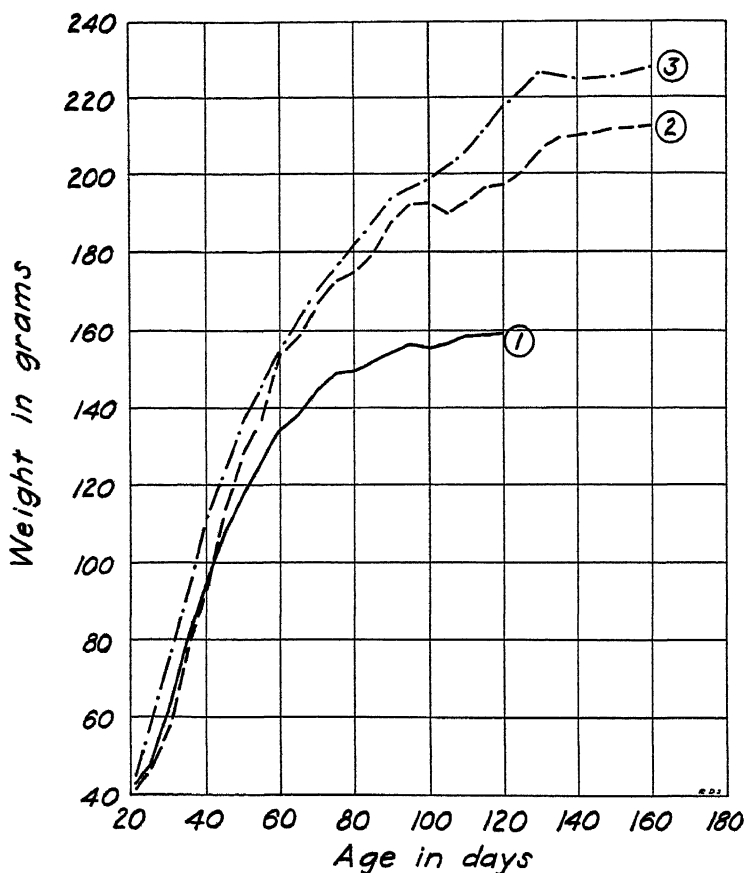


Fig.1 Growth of female rats on (1) fat-free diet, (2) the same diet with 50 mg. of ethyl linoleate daily, (3) stock diet XIV.

some did not ovulate at all. The positive controls showed a normal estrous rhythm. The ovulation data are given in table 1.

The skin symptoms of the 'fat-deficient' animals were, as they have always been in this laboratory (compare Evans and Lepkovsky, '32 a), comparatively mild. Only some scaliness and dandruff, and a tendency to lose hair on the face, particularly around the eyes, were observed. Several cases of hematuria were noticed, and the increased consumption of water was also evident, although no quantitative measurements were done.

Conduct of the tests. The animals which had reached a growth plateau were employed as test animals after they had shown a constant (within small fluctuations) or slightly declining weight over a period of at least 30 days. In the

TABLE 1
Incidence of estrus

	<i>Fat-low group (82 animals)</i>	<i>Positive controls (8 animals)</i>
Average number of vaginal cycles up to 120 days of age	5.8	14.0
Number of animals having the following number of cycles up to 120 days of age		
13-16 cycles	5	7
9-12 cycles	17	1
5- 8 cycles	26	0
0- 4 cycles	34	0

beginning of the tests the animals were on the average 156 days old (120 to 185 days) and weighed on the average 171 gm. (128 to 208 gm.). The test materials were fed together with the vitamin supplements on separate dishes daily (except Sundays) and were usually quickly consumed. Methyl ricinoleate and ethyl chaulmoograte, which rats refused to eat, were given with a small pipette directly into the mouth. A group of three rats was employed for each test. The curative effect was measured mainly by the intensity of the renewed growth, for this was easiest to record quantitatively, although the data concerning ovulation often gave valuable additional insight into the potency of the test substances. The disappearance of hematuria was also a good criterion of the effectiveness of a substance, but because only one-sixth

of the animals had exhibited a macroscopically noticeable hematuria before the tests, the occurrence of this symptom was too rare to have any practical significance. The test period lasted 50 days; the gain in weight and the number of vaginal cycles during this period were recorded, and the latter was compared with the number of cycles during an equally long period immediately preceding the test.

RESULTS AND CONCLUSIONS

The results of the tests as far as the growth response is concerned may be easily seen by inspecting the figures 2 to 6. These results are summarized in table 2 below, in which also the data concerning ovulation are given.

TABLE 2
Effects of the test substances on 'fat-deficient' rats

SUBSTANCE	DAILY DOSE	AVERAGE GAIN IN WEIGHT IN 50 DAYS (WITH PROBABLE ERROR)	AVERAGE NUMBER OF ESTROUS CYCLES DURING 50-DAY PERIOD		CONCLUSION
			Before test	During test	
Methyl linoleate	mg. 200	gm. 41.3 (\pm 7.4)	5.0	9.7	Producing maximal effect
Methyl linoleate	100	45.7 (\pm 3.0)	5.7	10.7	Producing maximal effect
Methyl linoleate	50	29.5 (\pm 4.5)	5.3	9.3	Effective
Methyl linoleate	25	20.3 (\pm 1.6)	5.3	8.0	Effective
Ethyl 12: 13-oleate, cis-trans	100	0.0 (\pm 2.7)	6.3	5.7	Ineffective
Ethyl 12: 13-oleate, cis	100	— 2.3 (\pm 2.4)	4.0	4.7	Ineffective
Methyl ricinoleate	100	— 10.3 (\pm 4.1)	3.0	1.0	Ineffective
Ethyl eruceate	100	2.7 (\pm 0.6)	7.7	6.0	Ineffective
Ethyl chaulmoograte	100	Animals died	—	—	Toxic
Ethyl chaulmoograte	50	— 2.3 (\pm 1.2)	9.3	5.7	Ineffective
Methyl arachidonate	100	43.7 (\pm 3.6)	2.3	7.7	Producing maximal effect
Methyl arachidonate	33	44.0 (\pm 4.3)	0.7	7.0	Producing maximal effect
Linoleyl alcohol	100	23.7 (\pm 0.2)	8.3	9.7	Effective

The experiments with methyl linoleate were done mainly in order to find the minimal dose giving the maximal growth response, thus furnishing an idea of quantitative effectiveness of this substance. Furthermore, it was hoped that these experiments would guide us in the matter of the dosage with other fatty acid preparations. It is, of course, evident that

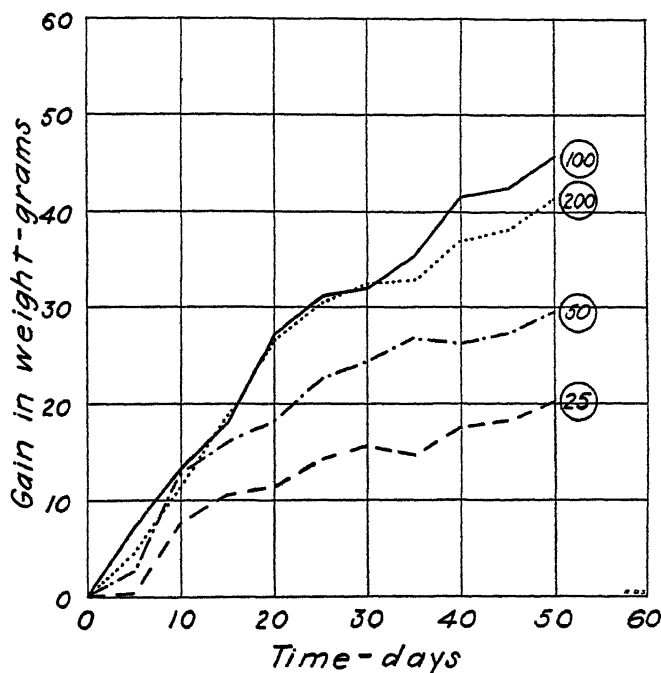


Fig. 2 The growth response of plateaued 'fat-deficient' female rats to the feeding of methyl linoleate. (The daily dose is given at the end of each curve.)

these results cannot claim a high degree of accuracy in the quantitative sense, because the groups of test animals were small, and, consequently, the probable errors of the means are comparatively large. It seems justified, however, to conclude from these experiments that about 100 mg. of methyl linoleate is needed to produce maximal growth in 'fat-deficient' female rats. The correct value is certainly between 50 mg. and 100 mg. and very probably closer to the latter.

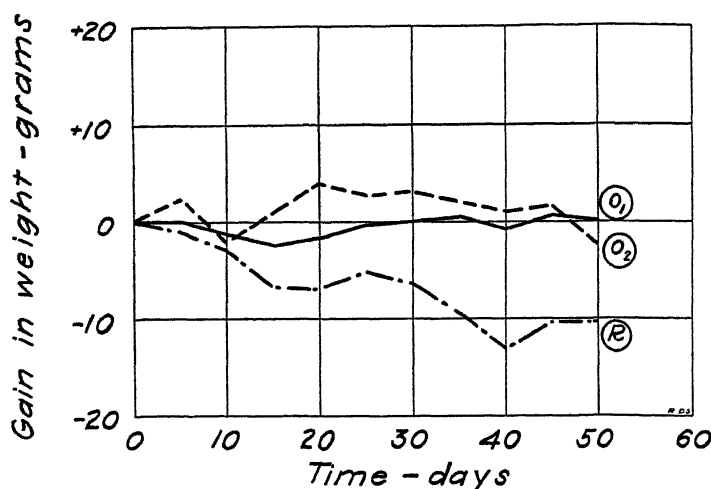


Fig. 3 The growth response of plateaued 'fat-deficient' female rats to the feeding of ethyl $\Delta^{12:13}$ -oleate and methyl ricinoleate. O₁, 100 mg. of ethyl $\Delta^{12:13}$ -oleate, cis-trans-mixture, daily. O₂, 100 mg. of ethyl $\Delta^{12:13}$ -oleate, cis-form, daily. R, 100 mg. of methyl ricinoleate, daily.

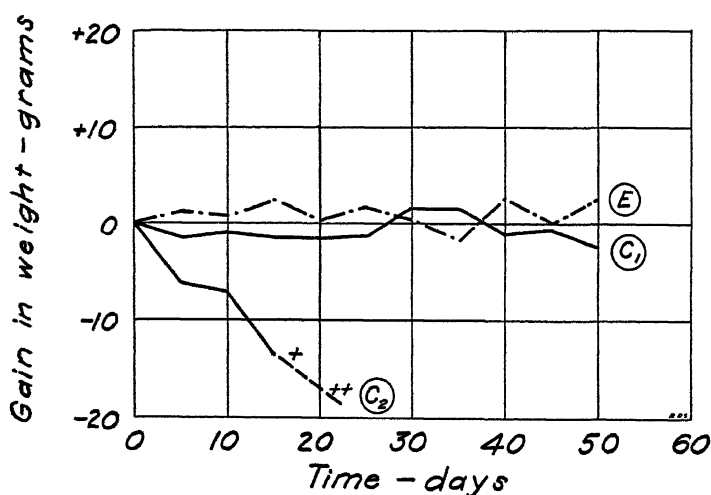


Fig. 4 The growth response of plateaued 'fat-deficient' female rats to the feeding of ethyl eruceate and ethyl chaulmoograte. E, 100 mg. of ethyl eruceate daily; C₁, 50 mg. ethyl chaulmoograte daily; C₂, 100 mg. ethyl chaulmoograte daily; + denotes the death of a test animal.

$\Delta^{12:13}$ -oleic acid ($\Delta^{12:13}$ -octadecenoic acid) was regarded as being particularly worth testing, because its only double bond is in the same position as the second double bond of linoleic acid (which structurally is $\Delta^{9:10,12:13}$ -octadecadienoic acid). It did not seem wholly improbable that the animal body, which is known to be capable of synthesizing ordinary oleic acid ($\Delta^{9:10}$ -octadecenoic acid), could also be capable of introducing the 9:10 double bond into the molecule of the $\Delta^{12:13}$ -acid, thus converting it into linoleic acid. In this case the $\Delta^{12:13}$ -oleic acid, of course, should be effective in curing the 'fat-deficiency' syndrome. The ethyl ester of this acid was, however, found to be without a trace of activity (fig. 3). This leads to the conclusion that the animal body, although capable of forming the 9:10 double bond, cannot introduce this double bond into the molecule of $\Delta^{12:13}$ -oleic acid.

Ricinoleic acid (12-hydroxy- $\Delta^{9:10}$ -octadecenoic acid) and erucic acid ($\Delta^{13:14}$ -docosenoic acid) were found to be completely devoid of curative effects (figs. 3 and 4).

Chaulmoogric acid (13- $[\Delta^{2:3}$ -cyclopentenyl] tridecanoic acid) at a level of 50 mg. of the ethyl ester daily was ineffective, and at 100 mg. level it showed a marked toxic action, killing the animals within 17 to 22 days. In view of the data of Emerson and Anderson ('34), who state that 30 cc. of ethyl chaulmoograte per kilogram when given as a single subcutaneous dose still represents a tolerated dosage, it would seem that in our experiments the substance displayed an unexpectedly high degree of toxicity. It is easy to understand, however, that the sensibility of 'fat-deficient' rats to chaulmoogric acid, a substance capable of causing renal irritation, may be considerably increased on account of the inferior condition of their kidneys. In fact, all our rats receiving the larger dose of ethyl chaulmoograte developed a severe hematuria during the tests.

The tests with arachidonic acid (an eicosatetrenoic acid, in which the positions of the double bonds are not definitely known) led to most interesting results (fig. 5). It proved

to be a very powerful agent in curing the 'fat-deficiency' syndrome. It was even more effective than linoleic acid. While about 100 mg. of methyl linoleate were needed to produce maximal growth in plateaued 'fat-deficient' animals, the same effect could be obtained with 33 mg. of methyl arachidonate. (The lag in the beginning of the 33 mg. curve

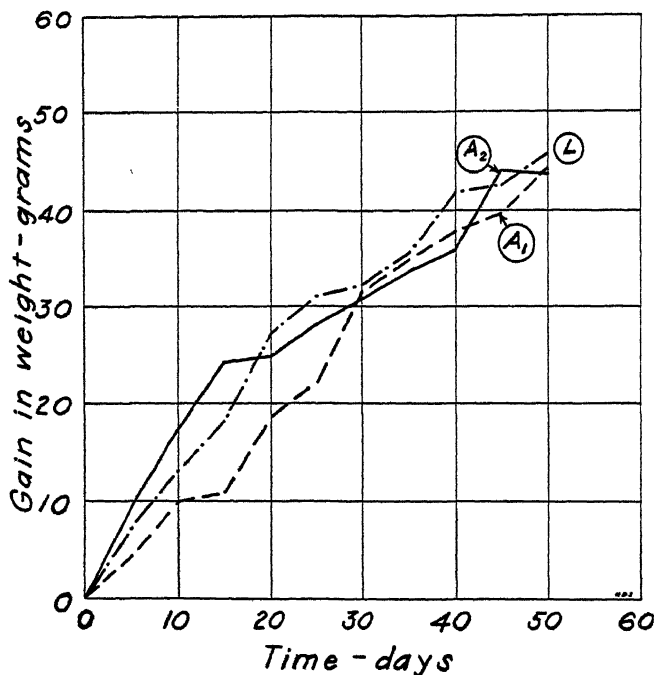


Fig. 5 The growth response of plateaued 'fat-deficient' female rats to the feeding of methyl arachidonate. A₁, 33 mg. of methyl arachidonate daily; A₂, 100 mg. of methyl arachidonate daily; L, 100 mg. of methyl linoleate daily—for comparison.

is probably not significant, because of the striking agreement of the curves in the later phases.) Whether even smaller doses of arachidonic acid would give maximal growth response must be left undecided. The tests reported here, however, justify the conclusion that arachidonic acid is at least approximately three times as effective as linoleic acid in curing the 'fat-deficiency' syndrome.

Linoleyl alcohol ($\Delta^{9:10,12:13}$ -octadecadienol) was tested mainly in order to find out whether the presence of a carboxyl group was essential for the effectiveness of a substance as a curative agent. In our experiments linoleyl alcohol exhibited unmistakable curative properties, although it did not equal linoleic acid in this respect (fig. 6). The activity of linoleyl alcohol was approximately one-fourth to one-third of that of methyl linoleate. Now, it is possible that the difficulty of the absorption of linoleyl alcohol may account for a part of this

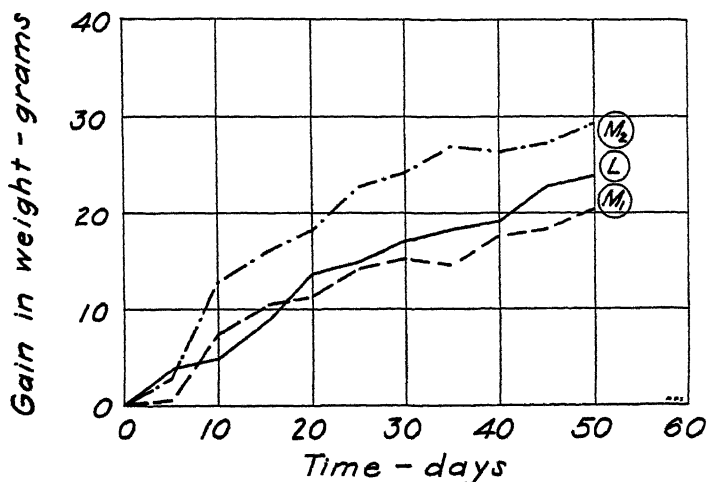


Fig. 6 The growth response of plateaued 'fat-deficient' female rats to the feeding of linoleyl alcohol (two levels of methyl linoleate included for comparison). L, 100 mg. of linoleyl alcohol daily. M₁, 25 mg. of methyl linoleate daily. M₂, 50 mg. of methyl linoleate daily.

difference. Channon and Collinson ('28) have demonstrated that the capacity of the rat to absorb higher alcohols like cetyl and oleyl alcohols is rather limited. In their experiments the absorption of oleyl alcohol by rats was on the average only 46 mg. daily. If this is also the case with linoleyl alcohol, the above results do not give a quantitatively correct picture of the effectiveness of this substance.

In conclusion it is perhaps worth while to summarize the evidence now available as to the curative effects of different

substances in the 'fat-deficiency' disease and to see if any general conclusions could be drawn. Including the fatty acids tested previously, we obtain the following classification:

<i>Effective</i>	<i>Ineffective</i>
Linoleic acid	Ordinary oleic acid
Linoleyl alcohol	$\Delta^{12:13}$ -oleic acid
Linolenic acid	Erucic acid
Arachidonic acid	Ricinoleic acid
	Chaulmoogric acid
	α -eleostearic acid

Now what are the particular features in the molecule which make a substance capable of curing the 'fat-deficiency?' Curative potency is undoubtedly linked in some way with double bonds. Is it the number of these which is most important or do their positions play the decisive role? Five different fatty acids with one double bond have thus far been tested and none of them has exhibited any activity whatever. They represent sufficiently different types of fatty acids (ordinary oleic and $\Delta^{12:13}$ -oleic acids with double bonds in different positions, ricinoleic acid with a hydroxy group, chaulmoogric acid with a cyclopentene structure, and erucic acid with 22 carbon atoms) to enable us to conclude with fair probability that fatty acids with a single double bond are inactive. At least two double bonds would appear to be necessary. But on the other hand, the need of the animal body cannot be simply for fatty acids with two or more double bonds, for α -eleostearic acid ($\Delta^{9:10, 11:12, 13:14}$ -octadecatrienoic acid) is inactive. The double bonds must evidently occupy certain positions. Both linoleic and linolenic acids possess double bonds in the positions 9:10 and 12:13, the latter acid has an additional double bond at 15:16. The last-mentioned double bond cannot, however, be of any importance, for linoleic acid is already, as Burr, Burr and Miller showed, quite as effective as linolenic. Thus the double bonds 9:10 and 12:13 are apparently the features which are responsible for the curative properties of an 18 carbon atom fatty acid. The carboxyl group is not essential; it may be changed for an alcohol group, although with some loss of potency.

The superiority of arachidonic acid as a curative agent to any other substance thus far tested would suggest the tentative hypothesis that the need of the animal body might be primarily for this acid. The wide occurrence of this acid in various tissues as a component of physiologically important substances (phospholipids) would also support the notion of its indispensability. Linoleic acid and the other 'effective' substances could perhaps be beneficial solely on the account of their conversion into arachidonic acid in the body.

SUMMARY

1. The effectiveness of the following substances to cure the 'fat-deficiency' disease in rats was tested for the first time: erucic acid, ricinoleic acid, $\Delta^{12:13}$ -oleic acid, chaulmoogric acid and linoleyl alcohol. Arachidonic acid was retested, and the effects of different levels of linoleic acid were estimated. The fatty acids were fed in the form of their methyl or ethyl esters.

2. Erucic, ricinoleic, $\Delta^{12:13}$ -oleic, and chaulmoogric acids proved ineffective.

3. In experiments conducted with four levels of linoleic acid, it was found that the maximal growth response in plateaued 'fat-deficient' females ensued when approximately 100 mg. of methyl linoleate were fed daily; twice this amount did not further improve growth, whereas half of it was patently inadequate.

4. Linoleyl alcohol showed some curative properties, although it was less effective than linoleic acid.

5. Arachidonic acid was found to be a powerful curative agent; it produced maximal growth response when 33 mg. of the methyl ester were fed daily, and was hence very definitely superior to linoleic acid in this respect.

The author is deeply indebted to Prof. Herbert M. Evans for suggesting this problem, for the generous provision of facilities in his laboratory, and for his interest and help during the course of the work.

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THE INFLUENCE OF HYDROGENATION AND OF YEAST IN COUNTERACTING COD LIVER OIL INJURY IN HERBIVORA, AND THE INFLUENCE OF SALMON OIL ON MILK FAT SECRETION ¹

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ONE FIGURE

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In an earlier report (McCay and Maynard, '35) the effect of cod liver oil, shark liver oil and salmon oil upon the composition of blood and milk was discussed. From this earlier study, the possibility was suggested that the constituent of cod liver oil which produces the decrease in the milk fat percentage might be the same as that responsible for the development of muscle lesions in Herbivora.

Hilditch and Thompson ('36) found that feeding cod liver oil to a cow increased the secretion of the highly unsaturated fatty acids of the C20-22 series into the milk fat. Since cod liver oil is rich in this series and shark liver oil is poor, it might seem that these fractions might be responsible for producing the heart lesions in Herbivora, as well as decreasing the secretion of milk fat. On the other hand, salmon oil is rich in this same series of unsaturated acids but fails to react upon the lactating cow with the constancy of cod liver oil.

In our earlier studies it was found that the active factor or factors for the production of muscle lesions and for lowering the secretion of milk fat were found in the saponifiable fraction of cod liver oil. This suggested that the activity might be due to some of the unsaturated fatty acids. To test

¹ Reported before the Rochester meeting of the A.C.S., September, 1937.

this hypothesis further, hydrogenation was employed as a means of partly saturating the double bonds of these unsaturated acids.

HYDROGENATED COD LIVER OIL² AND MILK FAT SECRETION

In a preliminary trial six cows were divided into two groups of three each. The first group was fed cod liver oil at the rate of $\frac{1}{2}$ cc. per day per kilo of live weight, while the second group was fed a similar amount of cod liver oil hydrogenated to the point where it still melted below 53°C. This experiment included a preliminary period before the oil feeding and one following to determine the level of fat secreted by each cow before and after, as well as during the oil feeding.

The three cows fed the ordinary cod liver oil responded in the usual manner with a decrease in the percentage of fat in the milk and an increase in the iodine number of the milk fat. In the case of those fed the hydrogenated oil, there was no change in two cases, but a sharp drop during the second week of oil feeding in the case of the third cow. However, the milk fat percentage of this cow never recovered during the 3 weeks following the oil feeding. This phenomenon is contrary to that observed uniformly after feeding cod liver oil. Ordinarily in cod liver oil feeding the fat per cent starts to recover as soon as the oil feeding stops and is usually back to the normal level for the individual at the end of 2 or 3 weeks.

These findings indicated that the hydrogenation of cod liver oil altered it to the extent that it no longer affected the secretion of milk fat. A second experiment to test further the effect of hydrogenated cod liver oil upon the secretion of milk fat was then designed.

In this next study a large sample of cod liver oil was purchased in the market. Half of this was hydrogenated so that the iodine number fell from 160 to 69. The melting point of the hydrogenated oil was below 53°C. The vitamins were probably partly destroyed because of the high temperature

² The cod liver oil used in these studies was hydrogenated through the courtesy of H. G. Miller of the Procter and Gamble Company.

of the hydrogenation, but in the present case the oil was not employed for a vitamin supplement.

The purpose of these new experiments with this hydrogenated product was first to determine if hydrogenation altered the component responsible for reducing the milk fat in a lactating cow.

Numerous studies have shown that the response of the lactating cow to the feeding of cod liver oil is remarkably constant. If a cow is fed $\frac{1}{3}$ cc. of cod liver oil per kilo of live weight per day, the decline in the percentage of milk fat usually begins within the first week and within 2 to 3 weeks the fat that is secreted into the milk is markedly below the normal for the individual and the basal ration. Because of the constancy of this reaction few test animals need be used.

For the present study six cows were so selected that none would be more than 6 months with calf at the end of the oil feeding. In order to avoid environmental factors, these were divided into two groups of three each. They were fed the regular winter ration for a period of 2 weeks in order to establish the fat percentage of the milk of each individual. The members of group 1 were then fed $\frac{1}{3}$ cc. of cod liver oil per kilo of body weight daily while group 2 was fed the same amount of hydrogenated oil. This oil feeding was continued for 3 weeks. Both groups were then allowed a rest period of 3 weeks with no oil. The oil feeding was then reversed so that group 1 was fed the hydrogenated product and group 2 the original oil. After this second period of oil feeding for 3 weeks, the cows were turned to pasture with no further oil feeding. An aliquot of milk was taken at each milking. The fat was determined at weekly intervals upon these pooled aliquots by the Roesse-Gottlieb method. The iodine number was run by the Hanus method.

The results of this experiment are summarized in figure 1. The data are plotted in mean values for each group of three cows, for the sake of simplicity. The curves are conclusive in showing the downward trends of the fat percentage during the feeding of the oil and the normal trends during consumption of the hydrogenated product. The mean fat percentages

for each cow during each week of the study are given in table 1, to illustrate the responses in each case. In every one of the six cows, the cod liver oil produced the usual effect in lowering the fat percentage of the milk and in elevating the iodine number. The hydrogenated cod liver oil had no effect upon the secretion of the milk fat and little if any on the iodine number of this fat. Furthermore, the animals

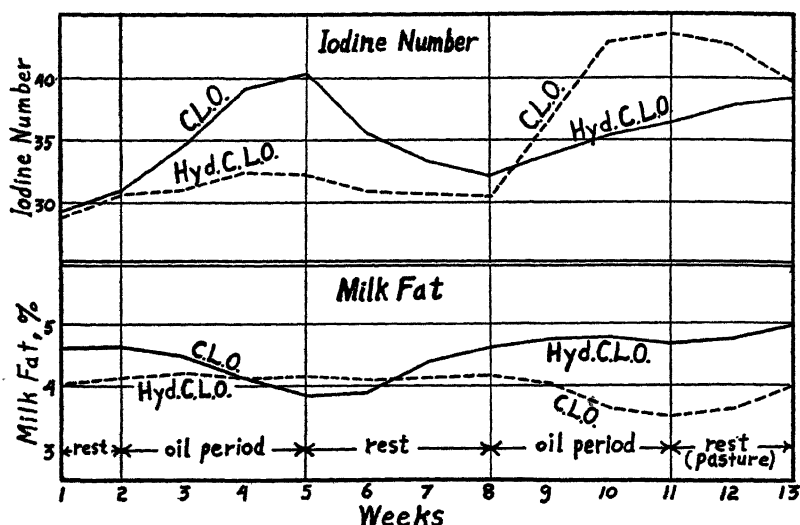


Fig. 1 The effect of feeding cod liver oil and the hydrogenated product to lactating cows. The ordinary decreases the secretion of milk fat while the hydrogenated does not. The former also increases the iodine number of the milk fat much more than the latter.

TABLE 1
Mean fat per cent in the milk of each cow during each week

COW	HERD RATION			COD LIVER OIL			HERD RATION			HYDROGENATED COD LIVER OIL			PASTURE	
G	4.60	4.58		4.59	3.98	3.96	3.98	4.49	4.51	4.93	4.84	4.66	4.67	4.83
F	5.60	5.70		5.60	5.57	4.87	5.03	5.54	5.74	5.92	5.86	5.50	5.78	6.62
C	3.70	3.75		3.38	2.90	2.68	2.73	3.24	3.62	3.90	3.70	3.82	4.01	3.87
				HYDROGENATED COD LIVER OIL						COD LIVER OIL				
T	4.30	4.69		4.69	4.50	4.83	4.71	4.55	4.58	4.63	4.21	4.20	4.19	4.46
L	3.89	4.04		3.97	3.87	3.85	3.76	3.82	4.13	3.75	3.43	3.25	3.70	3.86
E	3.93	3.87		4.12	3.82	3.95	3.76	4.08	3.90	3.80	3.35	3.07	3.07	3.54

regularly exhibited their distaste for the regular oil but no reaction toward the hydrogenated product.

THE PRODUCTION OF MUSCLE LESIONS

In an earlier paper the production of Agduhr's muscular degeneration by cod liver oil was described and the literature in this field reviewed (Madsen et al., '35). In the course of a number of years devoted to this problem, it has been found that guinea pigs fed synthetic diets are very sensitive test animals. They respond in a relatively short time. Their reaction to the feeding of cod liver oil is much less uniform than that of the lactating cow, however, probably because the feeding of synthetic diets to *Herbivora* has not been sufficiently developed.

To compare the toxicity of the two forms of cod liver oil previously fed to cows, ten guinea pigs were divided into two groups by age and sex. The following basal diet was used. This consists of casein 15, sucrose 15, starch 35, mineral mixture 4, regenerated cellulose 15, agar 5, irradiated yeast 7, and tomato juice (2 cc. per day per 100 gm. of body weight).

Group 1 was fed this basal diet mixed with 4% of the cod liver oil, while group 2 was fed this same basal with 5% of the hydrogenated product. The higher level of the latter was used to compensate for any likely increased excretion in the feces and to make the test more rigid. To the hydrogenated oil enough carotene was added to provide 100 units of vitamin A per day to compensate for probable loss in this factor during processing. For histological study, the gastrocnemius and vastus quadriceps muscles were fixed in Zenker's solution and treated in the usual manner. Experience has shown that these muscles reflect the action of the cod liver oil in such experiments.

The results of this study are summarized in table 2. The number of animals is small but the trend is clear. The tendency of the guinea pigs to die and to exhibit muscle lesions if they live over 2 weeks when fed cod liver oil is evident, in agreement with our previous findings. On the

other hand, it is questionable if any of the animals fed the hydrogenated product developed muscle lesions. Furthermore at the end of 2 months there were no animals alive in the cod liver oil group, while three were alive in the other. The body weight data which are not reproduced here indicated the growth and development of those fed hydrogenated cod liver oil and the failure of the other group. The beneficial

TABLE 2

Post mortem data on guinea pigs fed regular and hydrogenated cod liver oils

GROUP	ANIMAL NO.	DAYS OF EXPERIMENT	MANNER OF DEATH	LESIONS, VASTUS QUADRICEPS AND GASTROCNEMIUS MUSCLES ¹	NOTES
I Regular cod liver oil	2	7	Died	No sections	Refused to eat
	5	16	Died	+ (slight)	Hard dry feces—impaction
	3	18	Died	+ to ++ (areas)	
	4	31	Died	+++ to ++++	
	1	57	Killed	+++	Moribund—could not walk
II Hydro- genated cod liver oil	7	21	Died	Negative ²	Diarrhea
	9	24	Died	Isolated degenerated fiber? ²	Impacted large intestine
	6	60	Killed	Negative	Slightly mottled liver
	8	60	Killed	Negative	Normal
	10	60	Killed	Negative	Normal

¹ The lesions are classified as + to ++++ according to degree of injury, as in our previous publication (Madsen et al., '35).

² These tissues had areas that were not strictly normal, but the very slight changes shown may have been due to difficulties in fixing.

effect of the hydrogenation seems clear. Much longer periods of observation would be needed to decide whether it entirely eliminates the toxic factor.

THE FAILURE OF YEAST TO COUNTERACT THE EFFECT OF COD LIVER OIL UPON MILK FAT AND THE FAILURE OF SALMON OIL IN SHOWING THE REACTIONS OF COD LIVER OIL

From the early studies of Agduhr ('28) as well as ourselves (Madsen, McCay and Maynard, '35), it is evident that the composition of the basal diet as well as the cod liver oil is responsible for the production of degenerated muscles. This

has been amply confirmed in later work both in our laboratory and by Madsen ('36). The literature furnishes some indication that yeast can partly counteract the toxic action of cod liver oil in the development of muscle lesions, but furnishes no data concerning any interaction between cod liver oil and other factors in the diet of the cow. For this reason experiments were run with a group of cows to determine if there were any indications that when yeast and cod liver oil were both fed the secretion of milk fat might fail to decrease in the usual manner.

In this experiment six cows were divided into two groups of three each. One group was fed cod liver oil at a level of $\frac{1}{3}$ cc. per kilo of live weight and as much yeast³ as each individual could be induced to eat. This yeast was first mixed with the grain mixture and later with the silage. By this means each cow was fed from $\frac{1}{2}$ to 1 pound daily.

Since cod liver oil alone is very consistent in reducing the milk fat, it was considered more profitable in this preliminary trial to use another fish oil as a control. Thus the other group of three cows was fed salmon oil at the same level as the cod liver oil, but received no yeast. This experimental period lasted for 3 weeks. From these preliminary experiments no evidence was obtained that yeast had any effect in counteracting the action of cod liver oil in lowering the secretion of milk fat. The fat percentage dropped in the usual manner and the iodine number of this fat increased. During the 3 weeks following the feeding of the oil and yeast the milk fat secretion returned to its earlier level, as if cod liver oil had been fed alone. No further studies were made with yeast since these preliminary ones had given no evidence of activity in counteracting the effect of cod liver oil.

The results with the salmon oil were conflicting. The milk fat in one case dropped suddenly the first week and then arose, in the course of the next two weeks, to the initial level. In a second cow the fat remained at a constant level for the first

³ The dried yeast was obtained through the courtesy of C. N. Frey of the Fleischmann Company.

2 weeks of the oil feeding and then dropped in a manner comparable to that caused by cod liver oil. In the case of the third cow the curve for the drop of milk fat resembled that which is obtained in almost every case of feeding cod liver oil to a lactating cow.

These results indicated, like our earlier studies with this product, that it either lacked the factor of cod liver oil or had it present at a much lower level. Therefore another experiment with salmon oil was run. In this case four cows were fed salmon oil as a supplement to their usual feed at a daily level of $\frac{1}{3}$ cc. per kilo of body weight. After a preliminary period, the oil was fed daily for 3 weeks. This in turn was followed by a rest period with no oil. The results were conflicting again. One cow was unaffected and the fat level of the milk remained unchanged. Two exhibited a slight lowering at the end of 3 weeks and for the fourth the curve resembled that for cod liver oil. All samples of milk fat tended to exhibit an increase in the value of the iodine number during the salmon oil feeding and this value dropped back to the normal for the individual and ration in the course of the first week after the oil feeding stopped. No explanation for these conflicting results in milk fat secretion could be found in such factors as disturbance of the cows or volume of milk secreted.

No further studies were made with salmon oil but it is evident that higher levels than $\frac{1}{3}$ cc. per kilo must be employed. Since salmon oil is from the body as well as from the liver, it is possible that the fish liver contributes the active factor rather than the fish body as a whole.

SUMMARY

Two experiments were run with lactating cows to determine the effect of feeding hydrogenated cod liver oil upon the secretion of milk fat. In contrast to the original oil, the hydrogenated product neither lowered the fat percentage of the milk nor significantly raised the iodine number of the milk fat. Limited evidence from feeding this hydrogenated

oil to guinea pigs also indicated that it does not produce muscle lesions over a period of 60 days, although such lesions are regularly produced with the oil before hydrogenation. The feeding of from $\frac{1}{2}$ to 1 pound of dried yeast daily with the usual amount of cod liver oil did not counteract the lowering of the milk fat caused by the oil in lactating cows. Two additional studies of the influence of salmon oil on milk fat secretion in cows indicated that this oil does not react like cod liver oil. If the injurious factor is present it is probably in a lower concentration.

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THE EFFECT OF MELTING POINT OF FAT UPON ITS UTILIZATION BY GUINEA PIGS

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For 50 years or more it has been recognized that one of the factors which determines the utilization of fat by an animal body is the melting point of this fat. Arnschrink (1889) was one of the early workers who classified fats into three groups upon the basis of their melting points and relative utilization within the body. Most of these early studies were made with dogs. Recent studies of Steenbock, Irwin and Weber ('36) indicate that the rate of digestion and absorption of different fats within the body of the rat differs during the first few hours, but after 12 hours the differences are questionable.

A few balance studies have been made with men to determine the relative utilization of fats of different properties. For the most part, however, the periods used in such studies have been very short. Thus in the recent work of Massatsch and Steudel ('35), 3-day periods were employed in comparing the utilization of lard and hydrogenated fat.

Few studies of fat utilization by species other than omnivora or carnivora have been made. In studying the passage of fat from the feed into the milk of goats Gogitdse ('04) concluded that higher melting fats were absorbed with difficulty in the case of goats.

In experiments with brook trout to determine the relative utilization of hard and soft fats, McCay and Tunison ('34) found that oils such as cottonseed or salmon oils were always better utilized than hard fats such as hydrogenated ones. In

the case of brook trout this might be anticipated since the processes of the body operate at a temperature of 8°C. These reactions in the body of the fish have been reviewed recently (McCay, '37).

To study this matter further exploratory experiments were made with rats and guinea pigs. Four adult rats were fed dry skimmed milk for a period of 10 days to establish the intake. In a following period of 10 days approximately 20% of the calories was replaced by cottonseed oil.¹ The last 5 days of this period the feces were collected. During the following

TABLE 1
Utilization of the fat in diets of skim milk milk and cottonseed oil and skim milk and hydrogenated oil by rats

	RAT NO.	FAT INTAKE MILK	(5 DAYS) FAT	FAT IN FECES (5 DAYS)	UTILIZATION
		gm.	gm.	gm.	%
Cottonseed oil	1	0.261	3.310	0.076	97.9
	2	0.194	3.128	0.113	96.6
	3	0.286	3.300	0.123	96.6
	4	0.267	3.152	0.052	98.5
Hydrogenated oil	1	0.260	3.011	0.165	95.0
	2	0.252	3.020	0.144	95.6
	3	0.308	3.470	0.134	96.5
	4	0.187	3.027	0.248	92.3

10 days hydrogenated fat² was fed similarly and the feces collected during the latter half of the period. The data from this experiment are summarized in table 1. These results indicate that the rat is able to use both fats quite effectively.

In the case of guinea pigs³ another technic was employed. A diet low in fat was first prepared by extracting a mixture of grain and ground alfalfa with isopropyl ether. This extracted mixture was then supplemented by the feeding of 10 cc. of tomato juice daily to each animal. Care was used to

¹ Wesson oil was the product used.

² Crisco was the product used.

³ These preliminary studies with guinea pigs were made by Mrs. L. Dudgeon and Miss M. Zoller.

moisten this grain mixture to insure adequate consumption. The experiment was divided into three periods of 11 days each. During the first, the low fat diet was fed. The feces were collected during the last 6 days of the period. During the next period the feed was mixed so as to contain 6% of cottonseed oil and the feces collected again during the last 6 days. In the final period the feed contained hydrogenated oil⁴ at the same level instead of the cottonseed oil. Three guinea pigs were used in these experiments.

The feces were preserved in alcohol then dried in vacuo and ground. Samples were then extracted with ether in the

TABLE 2

Relative utilization by guinea pigs of oil before and after hydrogenation

<i>Diet</i>	<i>Lipids in dry feces, per cent</i>
Basal (extd.)	0.96
	0.97
	0.80
Basal plus cottonseed	1.69
	1.85
	1.85
Basal plus hydrogenated oil	5.43
	5.86
	5.46

usual manner. Table 2 shows the variations in the fat content of the feces, although the weight of dried feces excreted was but little different in the various periods.

Further calculations indicated that in this trial about 90% of the oil was utilized and only about 60% of the hydrogenated product. These values indicate the differences clearly but are probably much less accurate than later ones that were obtained after a couple of years additional experience in feeding guinea pigs these special diets.

In order to explore this field further, a new series of preliminary trials with a number of different oils was run using essentially the same technics. Details will not be reported since the later experiments were similar and somewhat more

⁴ The hydrogenated oil used was Crisco.

accurate. In this second preliminary series were included not only cottonseed oil and its hydrogenated product but also shark liver oil, soybean oil, castor oil, palm kernel oil, hydrogenated cod liver oil, cod liver oil, olive oil, butter, coconut oil and peanut oil. These preliminary trials yielded some surprising results in addition to confirming the first findings, namely, that hydrogenated oils are not utilized to the same degree as lower melting ones. Castor oil not only seemed to have no cathartic action upon the guinea pigs but apparently was utilized as well as such oils as shark liver and peanut.

TABLE 3

Utilization by guinea pigs of various fats as supplements to a low-fat diet

<i>Fat supplement in diet</i>	<i>Fat in feces, per cent</i>	<i>Utilization, per cent</i>
Castor oil	1.25	96.2
Soybean oil	1.54	94.5
Olive oil	1.60	94.5
Coconut oil	1.69	94.0
Salmon oil	2.03	94.0
Cod liver oil	1.65	93.8
Neatsfoot oil	1.64	93.5
Peanut oil	2.33	91.8
Butter	2.58	91.0
Cottonseed oil	3.36	87.4
Corn oil	3.35	86.5
Mutton tallow	5.22	79.8
Lard	6.70	75.2
Hydrogenated cottonseed oil	8.42	73.8
Beef tallow	7.05	72.0

A third experiment was now run taking advantage of earlier experience in feeding the animals. Ten kilograms of the basal diet were first extracted and dried carefully to free it from isopropyl ether. Three healthy guinea pigs were selected that would consume the diets readily. Each lipid to be tested was incorporated to comprise 6% of the diet. This mixture was then fed for a period of 11 days. The feed intake was determined and the feces collected during the last 7 days of each period. Since these animals were maintained upon the diets for a long period of time, they were fed adequate supplements of an A-D concentrate and tomato juice, allowing 8 cc. of the latter per day.

The data from this series are summarized in table 3. The results are very similar to those of the two preceding experiments. The guinea pig seems unable to utilize higher melting fats as effectively as oils. In this series again the castor oil seemed to be well digested and absorbed. No evidence of a cathartic action was found.

No corrections were made for metabolic fat in these studies since the results are evident without such corrections and since calculations involving metabolic fat make the assumption that the excretion of fat into the intestine is the same during periods of low fat and of normal fat ingestion.

TABLE 4

Fecal lipids as determined by the Tidwell-Holt method and by the regular ether extraction procedure

<i>Oil supplement</i>	<i>Tidwell-Holt method, per cent</i>	<i>Ether extract method, per cent</i>
Castor oil	2.31	1.25
Olive oil	2.34	1.60
Cottonseed oil	3.75	3.36
Hydrogenated oil	9.15	8.42

In order to compare the amount of lipid extracted from the feces by ethyl ether and another procedure, a few typical fecal samples were run by the method of Tidwell and Holt ('36). The values of table 4 show the latter method yields somewhat higher values but the differences between the oils and higher melting fats show the same trend for both methods.

SUMMARY

In order to determine if the melting points of a fat in region of body temperature is important in influencing its utilization in Herbivora, a series of balance studies was made chiefly with guinea pigs. The following oils were fed, incorporated at a 6% level in a diet of alfalfa hay and grain which had been extracted previously with isopropyl ether: castor, soybean, olive, cocoanut, salmon, cod liver, neatsfoot, peanut, butter, cottonseed, hydrogenated cottonseed, corn, tallow and lard. The fecal lipid tended to be much higher after feeding

the higher melting fats. The higher melting fats are not so well utilized as the oils by the guinea pig in contrast to the rat. Even castor oil seems well absorbed.

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THE RELATION OF CELLULOSE AND LIGNIN CONTENT TO THE NUTRITIVE VALUE OF ANIMAL FEEDS ¹

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For the past 40 years, animal feeding stuffs have, for general description, been divided by proximate analysis into six fractions: moisture, ether extract, protein, ash, crude fiber and nitrogen-free extract. This analysis has been the common basis of the nutritional classification of feeds, particularly the three fractions (protein, fat and fiber) which have been used in feed control measures. Through an extension of this analysis to the feces voided by animals on controlled feeding tests, the usual digestibility coefficients for feeds are calculated. The significance of the specific values obtained, however, has frequently been over-estimated. This has been true especially of the crude fiber fraction.

In terms of feeding value, crude fiber is intended as a measure of the quantity of the fibrous, poorly digested material in the feed. On the other hand, one has but to recall the chemical procedure by which this fraction is isolated to understand that any relation crude fiber may have to the digestibility of a feed may be in part fortuitous. (For a full discussion of this subject, the reader is referred to the work of Norman, '35).

¹ The experimental work of this study was carried out largely in the Laboratory of Animal Nutrition, Cornell University, and excepting where otherwise noted, the data are taken from a thesis presented by E. W. Crampton in partial fulfillment of the requirements for the degree of doctor of philosophy.

That the digestibility of the dietary carbohydrate does not follow its partition into crude fiber and nitrogen-free extract with any marked certainty, especially in the case of roughages, is evident from table 1 calculated from digestion coefficients reported by Morrison ('37).

The published literature also contains ample evidence that the crude fiber of forages may be as well digested as the protein (Newlander and Jones, '32; Mitchell and Hamilton, '33; Morrison, '37). It would, therefore, seem that the justification for partition of the carbohydrate fraction of feeds into crude fiber as the poorly, and nitrogen-free extract as the highly digestible parts, may be open to some question.

TABLE 1
Relative digestibility of Weende crude fiber and nitrogen-free extract

KIND OF FEED	NUMBER AVERAGED	AVERAGE COEFFICIENT OF DIGESTIBILITY		PER CENT OF CASES WITH CRUDE FIBER SHOWING AS COMPLETE DIGESTIBILITY AS N-FREE EXTRACT
		Crude fiber	N-free extract	
		%	%	%
Dry roughages	110	52.4	59.5	39
Green roughages	61	63.5	76.3	20
Silages	25	58.2	64.6	28
Concentrates	88	53.3	78.5	10
All feeds	284	55.6	69.5	25

Furthermore, it is logical to believe that if a division of the carbohydrate fraction could be made into parts which were either biological or chemical units, the usefulness of the feeding stuffs analysis in predicting probable feeding value would be enhanced. The problem of such a partition resolves itself largely into a consideration of the chief constituents of the 'cell wall' carbohydrates—cellulose, hemicellulose and lignin.

Chemical and biological nature of lignin, cellulose and hemicellulose

Lignin occurs in plants chiefly as lignocellulose. There is support for the belief that substances of the glucosanxylan series are the forerunners of lignin, but neither its exact

chemical structure nor the manner in which it is combined with cellulose are fully understood. Its behavior in nutrition is likewise unsettled, different feeding tests yielding conflicting results (Dietrich and König, 1871; König and Becker, '18; Paloheimo, '25; Rogozinski and Starzewska, '27; Rubner, '28; Phillips, '29, '34; Fuchs, '36; Woodman and Stewart, '32; Prjanischnikow and Tomme, '36). Proof for or against its utilization by the animal is difficult to establish, for until its molecular structure is known, no criterion of the accuracy of a quantitative test for lignin is possible.

In preliminary tests of this study, and using chemical procedures to be later described, 97.8 and 99.3% of the dietary lignin were recovered in the feces of rabbits and a steer, respectively. Subsequent tests of the digestibility of certain dietary constituents of mixed pasture herbage clippings substantiated this finding with rabbits as seen in the following figures:

<i>Date of clipping of grass fed</i>	<i>Per cent dietary lignin recovered in feces ²</i>
May 12	97.56
June 3	95.18
June 20	93.39
July 9	100.32
July 9	103.67
July 24	96.87
August 20	102.73
Average	98.53

These data support the rather more general opinion that dietary lignin is not appreciably metabolized by animals.

Cellulose. Neither the chemical nature of cellulose, nor the steps by which it eventually yields products nutritionally useful to an animal, can be discussed here (see Pochon, '35; Woodman and Stewart, '28). Undoubtedly the extent to which such end products are produced may be influenced by a number of factors (Mangold, '34). Among them, the possible role

² Digestibility data from nutrition laboratory, Macdonald College and obtained for each clipping by pooling feed and feces figures for three rabbits fed individually on identical diets. The rabbit feeding was carried out by Mr. Robert P. Forshaw and the analyses of the feces by Mr. A. J. Sutherland, graduate assistants at Macdonald College. Their assistance is much appreciated and gladly acknowledged.

of lignin is of special interest in this study. Lignified plant tissues apparently are not attacked by alimentary bacteria (Woodman and Stewart, '32), perhaps because of a certain degree of antiseptic action of the lignin resulting from its phenolic nucleus (Hibbert).³

This obviously suggests that if lignification differs in degree between samples of forage either because of species or environmental conditions, the feeding value of such material may be changed as a result. This might, in part, explain the observed decrease in nutritive value of pasture grasses during seasons or parts of seasons when conditions are not favorable to rapid growth, but instead favor lignification of the plant tissues. On the other hand, it may not follow that, other factors constant, a decrease in digestibility of the feed will reflect an increase in total lignin, inasmuch as the manner of its deposition in the plant may be a factor of importance in this respect (Woodman and Stewart, '32).

Hemicellulose, according to Armstrong and Armstrong ('34), is perhaps most usefully visualized as consisting of a mixture of the components of incompletely formed woody substances containing, in association, true cellulose chains, xylan and polyglucuronates (see also Norman, '36). The usually accepted method of quantitative estimation (furfuraldehyde) is open to the criticism that hemicellulose, of animal forage at least, contains appreciable quantities of hexosans as well as pentosans. Biologically, this group of carbohydrates follows much the same paths of degradation as cellulose. However, there is some evidence (Schmidt-Ott, '36; Iwata, '35; Williams and Olmsted, '35) that they may be somewhat more completely utilized by the animal system, thus standing intermediate between cellulose and the easily soluble starches and sugars.

The large number of substances which appear logically to be classed as hemicellulose, together with the confusion which at present exists in the exact definition of this group, makes

³ Hibbert, H. Pulp and Paper Research Institute, McGill University, personal communication.

the possibility of a satisfactory chemical estimation rather remote. There appears to be no advantage for purposes of a feeding stuffs analysis in determining a part of the group, as, for example, pentosans, and still leaving an undetermined fraction. As an alternate plan, estimation by difference of the total hemicelluloses plus any other carbohydrates not cellulose, seems to be a more logical procedure and was adopted in this study.

Chemical estimation of lignin and cellulose

Chemical determination of lignin. Space does not permit a full discussion of the problems of lignin determination, and for such material the reader is referred especially to papers by Norman and Jenkins ('34), Goss and Phillips ('36), Cohen ('36), Cohen and Harris ('37), Waksman and Stevens ('30), Ross and Potter ('30), and Ross and Hill ('29). Consideration of these and other studies led us to the conclusion that at the present time some form of the '72% H_2SO_4 ' method is the most satisfactory for the quantitative estimation of the lignin content of animal forage. It consists in solution in concentrated H_2SO_4 of the sample which has been pretreated to remove fats, sugars and proteins which otherwise would interfere with the lignin values obtained.

The special problem, in the case of forage and animal feces, lies in the removal of the protein without a simultaneous removal of a part of the lignin. According to present information, lignin is soluble in varying degrees in dilute alkali (hot or cold), boiling water, and dilute mineral acid (1.25% H_2SO_4) at boiling temperature. Pretreatment by enzyme digestion, however, would seem to be a suitable possibility. Williams and Olmsted ('35), working with human diets and feces, proposed the use of pancreatin in a solution buffered at pH 8. In their proposal, cellulose and hemicellulose were determined on the filtrate remaining on removal of the lignin; but because some hemicellulose is removed by the alkaline pancreatin solution, a correction is made by running an additional sample of the diet with the pancreatin omitted.

Lignin values are taken from the sample digested with the pancreatin. But if lignin were isolated from both the enzyme and no-enzyme residues, any effect of the pretreatment on lignin would be seen. (Fats, starches and soluble sugars are removed by ether extraction and autoclaving previous to the enzyme treatment.)

When this procedure was tried with sheep and steer diets of grain and hay, the results in every case showed a smaller lignin value in the pancreatin treated sample, as indicated in table 2.

TABLE 2

Effect on lignin values of pretreatment of sheep and steer diets by pancreatin digestion

DESCRIPTION	LIGNIN (AS PER CENT OF AIR DRY SAMPLE)	
	With pancreatin	Without pancreatin
	%	%
Sheep diet—grain + alfalfa hay	4.76	5.40
Sheep diet—grain + timothy hay	4.21	5.78
Steer diet—grain + alfalfa hay	2.81	3.40

It seems probable that the long exposure of the samples (72 hours at 45°C.) in a solution buffered at pH 8 using NaOH as the alkali, may have dissolved some of the lignin and thus resulted in the lowered lignin values.

Another difficulty was also encountered in the use of the Williams and Olmsted procedure. It was found impossible in the case of the animal diets to effect complete solution in the concentrated H_2SO_4 of the enzyme digest residue. Subsequently, when a lignin balance was struck from a steer digestion trial, 25% more lignin was recovered in the feces than was consumed.

Pepsin, on the other hand, is active in acid medium; and it has not yet been shown that lignin is soluble in dilute mineral acid at temperatures at which this enzyme is active. The effectiveness of this enzyme in removing proteins was uncertain in view of studies by Horwitt et al. ('36) in which not more than 89% of the nitrogen was removable from spinach leaf by pepsin digestion. There was the possibility, however,

that the protein of the materials would be reduced by pepsin digestion to a level no longer seriously interfering with lignin determination. If the hypothesis is accepted that lignin is not utilized by the animal, the usefulness of the pepsin pretreatment and subsequent analytical steps would be indicated by a lignin balance trial. Such a test yielded the data given in table 3. The practically quantitative recovery of the dietary lignin obtained argues well for the procedure used.

TABLE 3
Digestibility of lignin by rabbits and a steer

ITEM		RABBITS	STEER
Dry matter	Consumed	228 grams	53.00 pounds
	In feces	79 grams	10.75 pounds
Per cent lignin	In feed	9.03%	5.56%
	In feces	25.48%	27.12%
Weight of lignin	Consumed	20.58 grams	2.95 pounds
	Voided	20.15 grams	2.92 pounds
Balance		0.43 grams	0.03 pounds
Per cent recovery		97.8%	99.3%

The problem of completely and rapidly dissolving the undigested residue in the strong acid was solved by Ross and Hill ('29) who found that lignified tissues would dissolve promptly (10 to 15 minutes) in 72% H_2SO_4 if first moistened with formalin. With the sample in contact with the acid for so short a time, the difficulty with the formation of substances from the carbohydrates (pentoses or hexoses) which might add to the lignin value is presumably largely avoided (Ritter et al., '32). The use of a granulating reagent (chloroform-acetic acid) in the precipitation of the lignin to hasten the time necessary for filtration was proposed by Ross and Potter ('30), and has been recommended by Baillie ('36), in a micro-technic. It appeared, therefore, that pepsin digestion of the ether extracted sample, followed by solution of the residue in 72% H_2SO_4 and subsequent precipitation of the lignin according to the Ross and Hill, and Ross and Potter procedures could be successfully used for lignin determination in animal feeds and feces.

Specifically, the procedure eventually adopted in this study is as follows: Place the oven-dry, ether extracted residue from a 1 gm. sample of feed (or feces) in a 50 cc. glass stoppered Erlenmeyer and add 40 cc. of a 2.0% solution of pepsin in N/10 HCl. Digest for 12 hours at 40°C., shaking frequently especially during the first 4 or 5 hours. Recover the non-digested residue by filtration through bolting silk and wash successively with hot water, hot alcohol, hot benzene, hot alcohol, and ether. Transfer the washed residue to a 100 cc. beaker, and remove the last traces of ether with mild heat. Moisten the residue with 4 cc. of 40% formaldehyde. Then add 4 cc. of 72% H_2SO_4 , and allow it to penetrate the sample (2 minutes). Add 6 cc. of concentrated H_2SO_4 and stir vigorously with a glass rod to aid in solution of the sample which should be complete in 10 to 15 minutes. Partially immerse the beaker in a cold water bath, if necessary, to prevent the temperature from rising above about 70°C. When dissolved, stir in 35 cc. of a granulating reagent consisting of a 1:6 mixture (by volume) of chloroform and acetic acid, and then pour the whole into 500 cc. distilled water in an 800 cc. beaker. Boil gently until the chloroform has been driven off (15 minutes), after which the solution should clear and the lignin settle in granular form. Filter on a Gooch with suction. Wash in not less than 200 cc. of 5% HCl. Dry at 110°C. and determine lignin by loss on ignition. This procedure has given reproducible results in the hands of two analysts.

Determination of cellulose. For a summary of the many methods which have been used for the quantitative estimation of cellulose, the reader is referred to papers by Kürschner and Hoffer ('31), and Kürschner and Hanak ('30). According to the former procedure, the sample was freed of non-cellulose, organic constituents by digestion with an alcohol-nitric acid reagent. The treatment involved boiling the sample with the reagent for two and sometimes three successive hour periods. In the latter procedure, acetic acid is substituted for the alcohol in the digestion reagent and the time of treatment reduced to 20 minutes.

Comparative tests of these two procedures resulted in practically the same values for cellulose from feces material, but lower values from certain feeds with the acetic acid- HNO_3 reagent, as is shown in table 4.

TABLE 4
Cellulose as per cent of moisture-free sample

	ALCOHOL- HNO_3	ACETIC ACID- HNO_3
	%	%
Steer feces	21.7	21.1
Rabbit feces	30.4	31.0
Steer feed (grain + alfalfa hay)	14.7	11.4
Rabbit feed (grass clippings)	22.4	22.3

A possible explanation of this might lie in a difference in the resistance of the cellulosan fractions of mature hay and grain as compared to those in immature grasses. Certainly, the $\text{CH}_3\text{COOH-HNO}_3$ mixture is the more powerful reagent. Incidentally, the results with the feces might be interpreted that the animal was able to remove most of the dietary cellulosans.

These results, together with its greater simplicity, led to the choice of the acetic acid reagent for the cellulose determination. It was found, however, that by using alcohol instead of water for the first washings to free the cellulose from the digesting reagent, this process could be carried out by centrifuging, which considerably facilitated the washing operations.

The details of the procedure used are as follows: Place a 1 gm., air dry sample in a 150 cc. round-bottomed, wide-necked flask fitted with a reflux condenser. Add 15 cc. of 80% acetic acid and 1.5 con. HNO_3 . Boil gently for 20 minutes. Transfer the sample and liquid to a 50 cc. centrifuge tube; add about 20 cc. of alcohol and centrifuge 10 minutes. Decant the liquid. Wash (in centrifuge tube) with alcohol. Transfer the residue (with aid of a stream of alcohol from a wash bottle) into an alundum crucible and wash successively with hot benzene, hot alcohol, and ether—using suction. Dry. Calculate cellulose

as loss on ignition. Our experience has shown this method to give reproducible results.

A test of a modified procedure for feeding stuffs analysis

This study was originally undertaken in connection with the problem of the nutritive value of pasture herbage where the

TABLE 5
Analysis of feeds and feces and apparent digestibility of diets

ANIMAL	PROCEDURE		ANALYSIS		PER CENT DIGESTIBILITY
			Per cent diet	Per cent feces	
Yearling Angus steer weight 850 pounds	Standard	Ether extract	6.59	7.61	76.6
		Protein (N \times 6.25)	12.19	18.73	68.8
		Ash	3.64	9.91	...
		Crude fiber	12.11	25.66	57.0
		N-free extract	65.47	38.08	88.2
Diet: equal parts alfalfa hay and grain	Modified	Ether extract	6.59	7.61	76.6
		Protein	12.19	18.73	68.8
		Ash	3.64	9.91	...
		Lignin	5.56	27.21	0.7
		Cellulose	11.42	21.12	62.5
Dry matter eaten: 53 pounds		Other carbohydrate	60.60	15.42	94.8
Dry feces: 10.75 pounds					
Mature rabbit	Standard	Ether extract	5.10	5.60	61.9
		Protein	23.75	13.15	80.8
		Ash	9.80	13.60	...
		Crude fiber	20.10	28.00	51.7
		N-free extract	41.25	39.65	66.7
Diet: pasture grass clippings	Modified	Ether extract	5.10	5.60	61.9
		Protein	23.75	13.15	80.0
		Ash	9.80	13.60	...
		Lignin	9.03	25.48	2.2
		Cellulose	22.35	30.40	52.9
Dry matter eaten: 228 gm.		Other carbohydrate	29.97	11.77	86.4
Dry feces 79 gm.					

lack of correlation between the 'standard' feeding stuffs analysis and feeding value as measured by the growth of rabbits had been especially noted. To test the value of the data obtained by modifying the standard method of analysis by the inclusion of the determinations for cellulose and lignin, analyses were made of the feed and feces from a digestion trial with rabbits and a steer. The feeds and feces were

analyzed for water, protein, ash, fat and fiber by the usual procedures and for cellulose and lignin by the methods previously outlined. The term 'other carbohydrates' was used to denote the residue obtained by difference. These data are presented in table 5.

These data suggest that the modified procedure makes a sharper distinction among the carbohydrates as regards their digestibility. The carbohydrates are partitioned into three fractions: a practically non-digested portion, lignin; a highly digestible fraction, other carbohydrates; and into cellulose which is both biologically and chemically a recognizable unit. The digestibility of the latter fraction by a given animal may be expected to vary inversely with the degree (or nature) of its lignification.

As a means of estimating their feeding value, the modified scheme of analysis here proposed would seem to offer distinct advantages over the standard procedure for the analysis of rations, especially of Herbivora. Further study is needed of the application of the methods proposed to various kinds of feeds, and of the correlation of the analytical data with the results of feeding trials. Such studies are now in progress at Macdonald College.

SUMMARY

Data are presented which show the limitations, as measures of digestibility and nutritive value, of the crude fiber and nitrogen-free extract partition of the carbohydrates in animal feeds. Based on studies of various procedures, methods are proposed for the determination of cellulose and lignin in feeds and feces. The usefulness of the inclusion of these determinations in feed analyses is shown by data obtained in digestion trials with rabbits and steers. The data indicate that, at least for Herbivora, a partition of the carbohydrate portion of a feed into lignin, cellulose and other carbohydrates may have more biological significance, and hence be of greater usefulness in predicting feeding values than the present division into crude fiber and nitrogen-free extract.

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EFFECT OF FEEDING HIGH LEVELS OF COPPER TO ALBINO RATS ¹

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It is now a generally accepted fact that copper is an essential element and that it functions specifically in the stimulation of hemoglobin formation in the presence of an otherwise adequate diet (Harrow and Sherwin, '35). Schultze, Elvehjem and Hart ('34) have called attention to the fact that in the rat, quantities of copper as low as 0.005 mg. per rat per day will give a distinct response, while the optimum level is between 0.01 and 0.05 mg. per day. Since it is commonly believed that copper is toxic, it seems desirable to ascertain the level at which this essential element begins to exhibit toxic effects.

Waddell, Steenbock and Hart ('31) observed that rats could tolerate much more copper when their daily dosage was mixed with a dry ration than when it was fed all in 1 dose in a small amount of milk. When the latter technic was used, levels of 4 and 16 mg. of Cu per day were very toxic for rats. Earlier work by Lindow, Peterson and Steenbock ('29) indicated that 5 mg. of Cu per day was tolerated by rats when the copper was mixed with a dry diet. Coulson, Remington and Lynch ('34) observed no toxic effects in rats fed a diet containing 527 parts per million of copper as CuSO_4 .

In the present work, copper sulfate was added to diets in amounts sufficient to give a copper intake of as high as

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40 mg. per day if food intake remained normal. Rats were fed ad libitum diets in which the copper was uniformly mixed with the ration. Thus the animals established their copper intakes voluntarily and the resulting intake was determined by accurate food consumption records. In addition to growth records, the animals were sacrificed and the blood, liver and spleen were analysed for copper.

EXPERIMENTAL

White rats 21 days of age were placed on control ration no. 351 (Kline, Elvehjem, Keenan and Hart, '34) minus cod liver oil. Throughout the experimental period vitamins A

TABLE 1

Average growth, food intake and copper intake of rats receiving high-copper diets

SERIES NO.	NUMBER ♂	NUMBER ♀	COPPER ADDED TO BASAL RATION	AVERAGE GROWTH IN 4 WEEKS	AVERAGE FOOD INTAKE	AVERAGE EXCESS Cu INTAKE
			<i>mg. per kg.</i>	<i>gm.</i>	<i>gm./rat/day</i>	<i>mg./rat/day</i>
8900	1	2	0	86	11.8	0
9000	4	1	0	126	11.8	0
8900	1	2	500	78	10.2	5.1
9000	3	2	500	89	10.1	5.05
8900	1	2	1000	51	8.2	8.2
8900	1	2	2000	5.0	4.9	9.8
9000	4	1	2000	6.0 ¹	5.9	11.8
8900	1	2	4000	—6.7 ²	1.9	7.6

¹ One died in fourth week.

² All died in first week.

and D were administered orally in the form of percomorph oil. After 1 week the rats were divided into groups and placed on diets containing 0, 500, 1000, 2000 and 4000 parts per million of copper as copper sulfate. The diet containing the highest level of copper was prepared by pipetting an exact amount of copper sulfate solution on the diet, which was then dried and pulverized. The other diets were prepared by mixing various proportions of the most toxic diet with the control diet.

Two series were run. In the first (S8900) the rats on 4000 ppm. of copper died in 1 week and this level was there-

TABLE 2

Copper content of blood, spleen and liver from rats receiving high-copper diets

	BLOOD, MILLIGRAMS Cu PER 100 GM. OF BLOOD	SPLEEN			LIVER		
		Dry weight, grams	Milligrams Cu per		Dry weight	Milligrams Cu per	
			Whole spleen	100 gm. dried spleen		Whole liver	100 gm. dried liver
Control							
8942 ♀	0.128	0.333	0.00462	1.39	1.60	0.0217	1.36
8974 ♀	0.103	0.226	0.00294	1.30	1.80	0.0305	1.69
8964 ♂	0.0887	0.168	0.00291	1.73	2.23	0.0287	1.29
9051 ♂	0.0762	0.227	0.00235	1.04	2.37	0.0385	1.62
9052 ♂	0.104	0.188	0.00246	1.31	2.40	0.0397	1.65
9061 ♀	0.105	0.157	0.000747	0.477	1.69	0.0309	1.83
9072 ♂	0.0882	0.254	0.00258	1.01	2.66	0.0343	1.29
9064 ♂	0.102	0.239	0.000813	0.341	2.48	0.0291	1.17
Average	0.0994	0.224	0.00243	1.07	2.15	0.0317	1.49
500 mg. Cu per kilogram ration							
8944 ♀	0.146	0.160	0.00368	2.30	1.82	0.287	15.8
8973 ♀	0.183	0.107	1	1	1.38	0.278	20.1
8961 ♂	0.146	0.222	0.00266	1.20	2.45	0.761	41.1
9053 ♂	0.105	0.323	0.00278	0.860	2.54	0.410	16.1
9054 ♂	0.124	0.251	0.00167	0.664	2.11	0.654	31.1
9063 ♀	0.146	0.157	0.00214	1.37	1.78	0.223	12.5
9074 ♀	1	0.110	1	1	1.17	0.057	4.93
9071 ♂	0.123	0.205	0.00246	1.20	2.12	0.595	28.1
Average	0.139	0.192	0.00257	1.27	1.92	0.408	21.2
1000 mg. Cu per kilogram ration							
8945 ♀	0.228	0.107	0.00345	3.24	1.52	2.27	150
8972 ♀	0.188	0.103	0.00270	2.63	1.50	2.47	165
8952 ♂	0.247	0.159	0.00373	2.35	1.34	0.769	57.3
Average	0.221	0.123	0.00329	2.74	1.45	1.84	124.0
2000 mg. Cu per kilogram ration							
8946 ♀		0.0810	0.00284	3.51	0.914	4.10	448
8962 ♀	0.239	0.0403	0.00611	15.2	1.14	4.46	431
8955 ♂	0.431	0.0674	0.00216	3.20	0.801	3.29	411
9055 ♂	0.210	0.0715	0.00125	1.75	1.03	4.39	427
9056 ♂	0.190	0.121	0.00269	2.23	0.928	4.55	490
9062 ♀	0.123	0.0846	0.00246	2.91	0.684	2.91	425
9073 ♂	0.217	0.0845	0.00611	7.23	0.915	3.85	420
Average	0.235	0.0786	0.00337	5.15 ²	0.916	3.94	436

¹ Too low to read.² If result on rat no. 8962 is not included the average is 3.46.

fore not used in the second series (S9000). The food consumption was determined by difference and extreme precautions were taken to eliminate spillage. The animals were weighed once a week. At the end of the fourth week the animals were anaesthetized with ether and blood was withdrawn for analysis using the method of Swanson and Smith ('32) to avoid any copper contamination and to obtain maximum amounts of blood. The rats were examined for gross pathology after which the livers and spleens were also removed for analysis. The blood and organs were analyzed for copper by the method of Fischer and Leopoldi ('34) using wet combustion with sulfuric and perchloric acids. The excess perchloric acid was removed with sodium sulfite. Whole spleens were analysed with 5 micrograms of copper added. Blood was analyzed in quantities ranging from 2 to 5 cc. Liver was analyzed using aliquots containing from 4 to 8 micrograms of copper. The growth, food intake, and copper intake of the various groups are shown in table 1. The results of the analyses are shown in table 2.

RESULTS AND DISCUSSION

It will be seen that the rats receiving 500 ppm. of copper injected about 5 mg. of copper daily and showed good growth and food consumption although both were slightly subnormal. The animals appeared normal in every respect. The copper analyses, however, revealed significant increases in the blood (39%) and spleen (19%) and very large increases in the liver ($14\times$). In the rats receiving larger doses of copper the growth and food intake were markedly depressed in all cases. The rats receiving 2000 ppm. in the diet made virtually no growth while the rats on 4000 ppm. lost weight rapidly and died. In the latter case the deaths are partly due to voluntary starvation since the actual copper intake was less than in the preceding groups. The food intake was insufficient to maintain the body weight as shown by Franke and Potter ('34). The results in the group on 4000 ppm. show the necessity of considering the effect of taste in determining the results obtained on a toxic diet. It is not possible to study copper

toxicity adequately by feeding the daily dosage in a small portion of the diet.

The application of the method of Fischer and Leopoldi for the analysis of copper in biological material has made possible the analysis of individual samples of blood, liver and spleen. The results obtained with blood and spleen are in marked contrast to those obtained with liver. Whereas the higher levels of copper increased the copper content of blood and spleen a maximum of five times, the copper content of the livers of the rats receiving 2000 ppm. was raised about 300 times. In the latter case the livers were noticeably jaundiced. The results obtained with 500 ppm. check quite closely with those of Lindow, Peterson and Steenbock ('29), and of Flinn and Inouye ('29). Our results are at variance with certain data published by Cunningham ('31). This author has reported an increase of only 1.5 to 3 times in the copper content of livers of rats fed 7.5 mg. copper per day, whereas the present work and that of Lindow, Peterson and Steenbock ('29) showed an increase of 14 to 20 times in the case of livers of rats fed 5.0 mg. copper per day. Our results illustrate the marked ability of the liver to take up extremely large amounts of ingested copper. Apparently the liver has a much greater ability than blood to retain copper.

These results indicate that copper does not begin to exhibit toxic effects in the case of the rat until at least 150 times the therapeutic dosage has been reached. Apparently the copper content of the liver can be increased as much as fourteen times over a short period of time without obvious damage. It is unlikely that this amount of copper will be ingested either in practical rations or in therapeutic doses. The liver is apparently the organ which plays a predominant role in determining the amount of copper which is to remain in the body.

It is difficult to conclude from this work the location of the toxic action of copper. However, it is interesting to note that the liver shows a large increase with each increase in intake while the blood shows the most pronounced increase between 500 and 1000 ppm., and this is the range in which copper becomes definitely toxic.

SUMMARY

1. White rats were fed ad libitum diets which contained 0, 500, 1000, 2000 and 4000 ppm. of added copper in the form of CuSO_4 .

2. The rats ingested voluntarily amounts of copper ranging from 5.05 to 11.8 mg. copper per day, although at 4000 ppm., food intake was so restricted as to result in partial starvation and rapid death.

3. Slight toxicity was observed on 500 ppm. with increasing toxicity on higher levels, as indicated by growth records.

4. The animals were killed after 4 weeks and the blood, spleens and livers were analyzed for copper. Whereas the copper content of the blood and spleens was increased a maximum of 2 to 5 times, the liver increased to a maximum of 300 times normal.

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STUDIES ON THE VITAMIN B₁ REQUIREMENTS OF GROWING CHICKS^{1, 2}

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THREE FIGURES

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Recent reviews (Hogan and Boucher, '33; Jukes, '37) on the nutritional essentials of the chick reveal a paucity of data on the quantitative requirement of this species for the anti-neuritic vitamin. Kline, Keenan, Elvehjem and Hart ('32), Elvehjem et al. ('32), Keenan et al. ('34), and Elvehjem ('35, '36) have reported that the chick may successfully be used for studies on this vitamin. The autoclaved natural grain ration (ration 242A) developed by these investigators (Keenan et al., '34) has afforded a rapid and simple means of assay for vitamin B₁ with the day-old chick. Since fairly normal rates of growth are obtained when the above ration is supplemented with adequate amounts of vitamin B₁, it was important to establish the requirement of this species using both the international standard and crystalline vitamin B₁ hydrochloride. There is some question about the manner of expressing the requirement since it is related both to body weight and carbohydrate metabolism. An expression per unit weight of feed is most valuable from a practical point of view. At some stages of growth the vitamin B₁ intake on this

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³Wilson and Company Fellow.

basis will be higher than the requirement but the method will measure the requirement at the most critical period. This procedure may readily be adapted to a determination of the antineuritic potency of vitamin B₁-containing substances.

EXPERIMENTAL

For these experiments, day-old White Leghorn chicks with an initial weight of about 35 gm. were obtained from the University Poultry Department. The chicks were placed in cages equipped with wire screen bottoms (two meshes to the inch) and suitable warmers. Each group usually contained four chicks. Water was supplied in porcelain cups daily. Weights were recorded weekly.

The basal vitamin B₁ low ration (ration 242A), modified by Keenan, Kline, Elvehjem and Hart ('34), was used throughout. Ration 242A has the following composition:

Autoclaved portion:

Ground yellow corn	57
Pure flour middlings	25
Crude domestic acid precipitated casein	12

Untreated portion:

Vacuum desiccated whole liver substance ⁴	2
Iodized salt (0.02% potassium iodide)	1
CaCO ₃ (precipitated)	1
Ca ₃ (PO ₄) ₂ (precipitated)	1
Cod liver oil	1

The preparations were tested for their antineuritic potency by substitution into the ration at various levels. In each case, the smallest level of material necessary to protect all the chicks in the group over a period of 5 weeks was determined. Any material fed on the percentage basis which protected the chicks for 5 weeks was found to continue to do so indefinitely. The rations were made up fresh weekly. In addition to the basal ration the chicks received 2 drops of halibut liver oil twice weekly to insure an adequate supply of vitamin A.

⁴ Wilson Laboratories, Chicago, Illinois.

While the rate of growth of the chicks during the first 2 weeks on experiment somewhat modified the minimum protective level, the vitamin B₁ requirement of the chick when expressed as per cent of the ration was remarkably constant. The results shown in figure 1 illustrate this fact. In five separate assays it was found that all of the chicks in groups 471, 490, 511, 540, and 553 were protected from polyneuritis

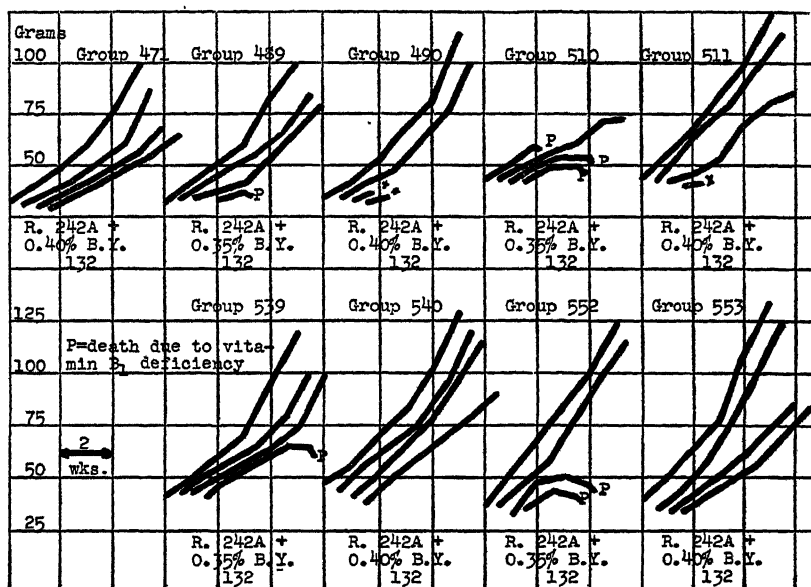


Fig. 1 Individual growth records of chicks fed ration 242A supplemented with 0.35 or 0.40% brewers' yeast 132 during a 5-week experimental period.

when the basal ration was supplemented with 0.40% brewers' yeast 132.⁵ When the level of yeast was reduced to 0.35%, one or more chicks in groups 489, 510, 539, and 552 died after exhibiting the typical symptoms of polyneuritis.

It has been found that chicks are able to elute the vitamin from the international standard acid clay adsorbate. The results obtained with different levels of the international

⁵ We are indebted to Dr. Harold Levine of the Premier-Pabst Corporation, Milwaukee, Wisconsin, for several generous samples of yeast.

standard are shown in figure 2. When the chicks were fed ration 242A supplemented with 0.175% international standard, all chicks in group 592 died, two showing polyneuritis, and two of the four chicks in group 604 died after exhibiting the

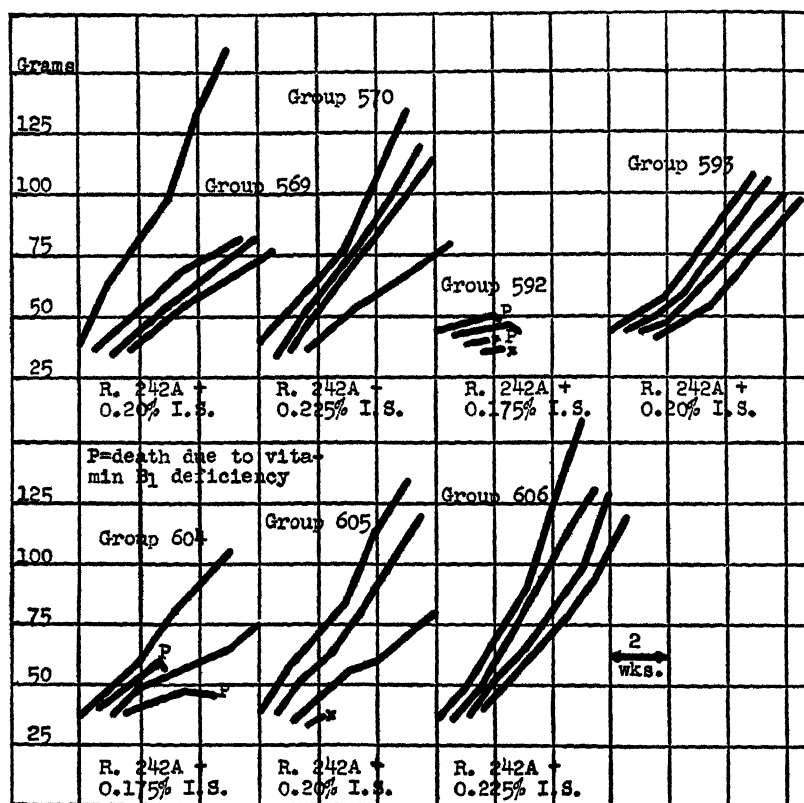


Fig. 2 Individual growth records of chicks fed ration 242A supplemented with 0.175, 0.200 or 0.225% international standard during a 5-week experimental period.

typical symptoms of polyneuritis. All of the chicks in groups 569, 593, and 605 were protected from polyneuritis when fed ration 242A supplemented with 0.20% international standard. An improved rate of growth resulted when the chicks were fed ration 242A supplemented with 0.225% international standard (groups 570 and 606). When the criterion for the

determination of the minimum protective level is taken to be protection from polyneuritis for all the chicks in the group, it is evident that the vitamin B₁ requirement of the chick is constant.

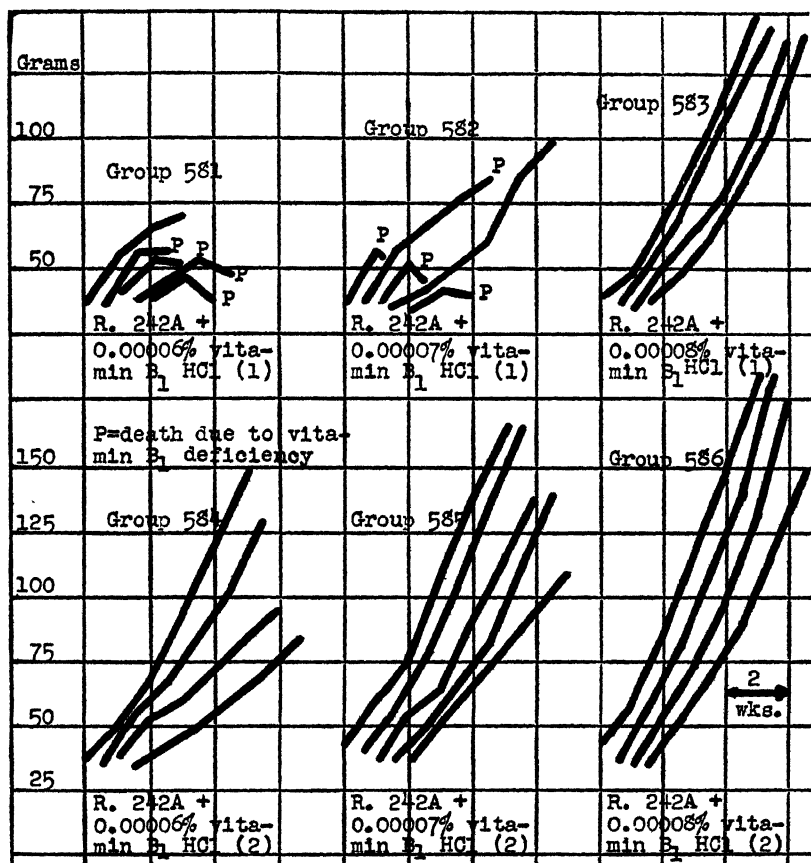


Fig.3 Individual growth records of chicks fed 60, 70 or 80 micrograms crystalline vitamin B₁ hydrochloride (samples 1 and 2) per 100 gm. ration 242A for a 5-week experimental period.

The requirement was also established in terms of the crystalline vitamin. Two accurately weighed samples⁶ of crystalline vitamin B₁ hydrochloride (synthetic) were dis-

⁶The vitamin B₁ samples were kindly weighed out by Mr. H. A. Campbell on a micro-analytical balance.

solved in 20% alcohol to give a final concentration of 100 micrograms per cubic centimeter. For feeding purposes, suitable aliquots were dried in vacuo on about 50 gm. portions of the autoclaved component of the ration. This was ground by hand before incorporation into the ration, care being taken to eliminate losses.

Typical results with the two samples of vitamin B₁ hydrochloride are shown in figure 3. Protection of the chicks from polyneuritis resulted when they were fed ration 242A supplemented with 80 micrograms of vitamin B₁ hydrochloride (sample 1) per 100 gm. of ration (group 583). Lower levels of this product did not protect all of the chicks from polyneuritis (groups 581 and 582). When the chicks were fed the basal ration supplemented with 60 micrograms of vitamin B₁ hydrochloride (sample 2) per 100 gm. of ration, complete protection from polyneuritis resulted (group 584). The wide variation in the growth rates of the chicks in this group was due to the fact that this was a borderline protective level. When the vitamin B₁ in the ration was increased slightly, growth was improved (groups 585 and 586).

DISCUSSION

The results in this paper, as well as other data obtained in our laboratory, indicate that the vitamin B₁ requirement of chicks based on unit weight of feed is constant. On the basis of the results obtained with the international standard it is seen that the requirement is between 20 and 25 international units per 100 gm. of ration 242A. Twenty international units protected against polyneuritis with several different groups of chicks on the specific basal used. Somewhat better growth resulted with 25 international units per 100 gm. of ration 242A. It is perhaps safer to assume that the normal requirement is about 25 international units since ration 242A is known to contain slight amounts of vitamin B₁. A slightly higher level may be necessary on a basal ration completely devoid of this vitamin.

A significant difference in the antineuritic potency was observed between the two samples of crystalline vitamin B₁ hydrochloride that were tested. Sample 1 was more active since 60 micrograms per 100 gm. of ration 242A protected the chicks from polyneuritis. Eighty micrograms of sample 2 per 100 gm. of ration 242A were required to protect the chicks from polyneuritis. The reason for the variation in antineuritic potency is not clear at the present time. Nevertheless, we feel justified in assuming that sample 1 yields a truer evaluation of the antineuritic potency of the crystalline vitamin.

The above data permit an evaluation of the antineuritic potency of brewers' yeast 132. Since 0.40% of brewers' yeast 132 was required to protect the chicks from polyneuritis, it follows that this yeast sample contained 50 international units per gram. Similarly, a comparison between the international standard and vitamin B₁ hydrochloride (sample 1) indicates that vitamin B₁ hydrochloride contained 330 international units per milligram. The ratio of the international unit to crystalline vitamin B₁ hydrochloride has been reviewed by Sampson and Keresztesy ('37).

The establishment of the values for the minimum vitamin B₁ requirements of chicks will greatly simplify the calculations of the vitamin B₁ content of food materials where chicks have been used for the assays. The results obtained with the chicks indicate that this method possesses certain advantages over the rat assay method. Less difficulty is experienced in the preparation of the basal ration for chicks than in the purification of material for synthetic rat rations. The data obtained with several different batches of chicks show that no difficulty is encountered with respect to storage of vitamin B₁ in the day-old chick. In addition it appears that the chick is more sensitive to small additions of the vitamin to the basal ration than the rat.

SUMMARY

1. The vitamin B₁ requirement of the chick as measured by the prophylactic technic is remarkably constant.

2. When ration 242A is supplemented with 20 to 25 international units of vitamin B₁ supplied by the international standard acid clay adsorbate the chicks are protected from polyneuritis.

3. Assays on samples of crystalline synthetic vitamin B₁ indicate that chicks are protected from polyneuritis when they are fed 60 micrograms of vitamin B₁ hydrochloride (sample 1) per 100 gm. of ration 242A. A second sample (sample 2) possessed lower antineuritic potency since 80 micrograms of crystalline vitamin B₁ hydrochloride per 100 gm. of ration 242A were required to protect the chicks from polyneuritis.

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THE EFFECT OF VARIED VITAMIN B INGESTION UPON THE APPETITE OF CHILDREN

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TWO FIGURES

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A recent editorial in the Journal of the American Medical Association ('37) calls attention to the point that additional data are necessary in order to determine the optimum vitamin B requirement of children.

Studies by Gaynor and Dennett ('34), Morgan and Barry ('30), Summerfeldt ('32), Ross and Summerfeldt ('35) and Poole, Hamil, Cooley and Macy ('37) would seem to indicate that an increase in vitamin B ingestion is frequently beneficial to infants and children. Knott ('36) reported data to show that preschool children retained increasingly greater amounts of vitamin B as the level of vitamin B in their diets was increased. In commenting on Knott's results, Sherman and Sherman ('37) question whether higher intakes of vitamin B are optimal because of the danger of 'forcing' growth.

Since the conclusions of Gaynor and Dennett were based chiefly on clinical evidence, and since Morgan and Barry, and Ross and Summerfeldt used only gain in weight as a criterion, the present study was undertaken to show the quantitative effects upon appetite of different levels of vitamin B intake when continued over a period of time.

EXPERIMENTAL PROCEDURE

Subjects. Two groups of children have been studied. The first group consisted of thirty-two children in the Country Home for Convalescent-Crippled Children. The majority of these children were convalescing from either tuberculous bone lesions or osteomyelitis, but at the time of this investigation their physical condition appeared to be fairly normal. Only those children were selected for the study who were able to live a normal child's life, including school and out-of-door play.

To contrast with this first group, a second group of twenty-two normal girls were selected from the Mooseheart Home for Child Training. These children were living together in one cottage, under the supervision of a matron and cook who assumed full responsibility for the care of the girls.

The physical status of both groups of children is itemized in table 1. Theoretical weights for most of the children were calculated by means of ¹ formulae from the Iowa Child Welfare Research Station. At the Country Home, sex, age, height and chest circumference (the latter being corrected for subcutaneous fat) were used to determine the theoretical weight. At Mooseheart, hip and knee widths were also used in the formula for the theoretical weight. The deviation of the average weight for each child from his theoretical weight is given in table 1.

Diets. At both the Country Home and at Mooseheart particularly well planned dietaries are employed. The children receive one serving of meat, at least three cups of milk, and two servings each of fruit and vegetables besides potatoes, in addition to simple desserts and refined and whole-grain cereals and bread each day. Raw fruits and vegetables are included several times a week, and eggs are given every other day. Throughout the study the above dietary was not changed at either institution, and supplements of vitamin B were made in addition to the regularly planned menus.

¹ Personal communication.

TABLE 1
Physical status of the children

GROUP I						GROUP II						
Child	Age	Sex	Average height	Weight		Child	Age	Average height	Weight		Time at the Home	
				Ave.	Dev. ¹				Ave.	Dev. ²		
	<i>years</i>		<i>cm.</i>	<i>kg.</i>	<i>kg.</i>		<i>years</i>	<i>cm.</i>	<i>kg.</i>	<i>kg.</i>	<i>years</i>	<i>mos.</i>
C.J.	5	F	93.8	15.7	+0.5	G.S.	9	122.2	23.7	-0.9	0	4
J.A.	6	F	108.	16.8	-1.7	E.F.	10	131.1	24.5	-0.4	7	3
R.J.	6	M	101.4	17.1	+1.0	B.P.	10	136.1	26.2	-0.4	8	6
L.T.	5	F	108.5	17.3	-1.3	C.C.	10	128.3	26.9	-0.1	1	0
E.B.	5	M	106.4	18.4	+0.3	B.W.	10	138.7	27.8	-2.2	7	1
J.M.	6	F	108.9	18.8	-1.8	D.W.	11	135.6	28.7	0.0	8	4
H.G.	6	F	109.3	19.7	-0.7	M.C.	10	128.5	29.2	-1.4	3	4
D.P.	6	M	111.1	20.3	-1.6	K.E.	10	136.1	29.4	-0.9	8	6
G.L.	5	M	111.1	20.7	-1.0	B.E.	11	140.7	30.8	-0.9	7	5
M.W.	10	F	119.3	21.	-1.7 ²	B.A.	10	140.0	31.7	-1.3	2	7
G.P.	10	M	122.4	21.6	-0.4	M.P.	11	133.6	31.7	+0.7	4	4
Ed.P.	7	M	116.6	21.8	+0.8	I.W.	11	135.1	32.	+0.2	0	1
A.O.	6	M	120.	22.1	-2.6	M.R.	11	137.9	32.1	-1.6	5	7
W.K.	8	M	118.1	22.2	+0.6	I.M.	11	146.3	33.1	-3.7	8	5
E.Ku	9	M	118.4	22.3	-0.3	F.D.	10	136.4	33.5	-1.6	8	10
M.R.	9	F	117.5	22.6	-0.2	P.W.	10	134.6	34.3	+0.3	6	9
G.W.	9	M	127.4	23.9	-1.4	M.D.	12	137.4	35.5	+1.2	0	7
J.T.	9	M	128.9	23.9	-3.3 ³	D.S.	11	141.0	37.1	-0.2	0	4
A.S.	7	F	116.6	24.1	+2.8 ²	A.G.	11	150.0	38.9	-0.8	6	9
M.L.	9	M	128.7	25.0	-0.6	S.B.	12	153.4	39.1	-1.3	0	3
J.S.	7	M	132.	25.1	-3.5 ²	A.P.	11	145.5	40.9	+2.1	6	3
L.S.	8	M	128.9	25.7	-2.7	E.G.	11	152.1	43.2	+2.1	6	9
T.O.	9	F	126.0	26.2	-0.6	J.B.	11	143.8	45.8	+6.4	0	3
T.L.	8	M	129.5	26.2	-2.5							
J.B.	10	M	134.5	26.7	-3.7 ²							
E.S.	9	M	130.5	27.2	-0.9 ²							
E.Ka	9	M	133.4	27.3	-2.3							
D.H.	9	M	130.4	27.7	-0.4 ²							
B.G.	9	M	130.2	30.5	+2.4 ²							
C.O.	11	F	136.	30.6	-4.0							
L.L.	10	M	136.3	30.7	-3.7							
D.M.	10	M	135.	33.	+0.2							

¹Theoretical weight calculated on basis of sex, age, height, and chest (corrected for fat).

²Theoretical weight calculated on basis of sex, age, height only.

³Theoretical weight calculated on basis of sex, age, height and body form.

Organization. The children at the Country Home were divided into three groups. During the first period group 1 received at breakfast 15 gm. of a refined wheat cereal²; group 2 received 15 gm. of a whole wheat cereal; and group 3 received the whole wheat cereal fortified with 3.3 gm. of a wheat germ preparation³ which had been stabilized by removal of fat and moisture. At the end of the first 8 weeks, and again at the end of the second period, the groups were rotated so that each group received each of the three different cereals. These three periods served as a control for the remainder of the investigation. During a fourth period the children were given free choice of as much breakfast cereal as they desired (the kind of cereal being changed from day to day), and received in addition a supplement of 1 teaspoonful of the stabilized wheat germ at each meal (making a total of 20 gm. of wheat germ daily). During the two final consecutive periods of 4 weeks each, each child received daily 455 micrograms of crystalline vitamin B.⁴ The stabilized wheat germ was given at each meal in a small amount of milk. The crystalline vitamin B was put in solution and added to the milk once a day during the first 4 weeks, and three times a day during the second 4 weeks of the final period.

At Mooseheart the investigation was divided into four periods, during the first and third of which the children received no supplement, while stabilized wheat germ was given in the second period and crystalline vitamin B⁵ during the fourth period. The first two periods were of 3 months duration each, the last two of 4 weeks each.

Methods. To determine food consumption of the children, sample servings of each food offered were weighed at each meal. Standard measuring devices were then used in serving

² The refined malted wheat cereal was supplied through the courtesy of the Campbell Cereal Company, Minneapolis, Minn.

³ The stabilized wheat germ, VioBin, was furnished by the VioBin Corporation, Chicago, Ill.

⁴ Natural crystalline vitamin B was kindly supplied by Merck and Company.

⁵ Synthetic crystalline vitamin B was supplied in the form of 0.1 mg. tablets of 'Betaxin' through the courtesy of the Winthrop Chemical Company.

each child so that all portions served would correspond to the weighed samples. At the Country Home complete records were kept at every meal of the number of servings of each food consumed by each child. Once a week the records were summarized and the average grams of food consumed daily were calculated. In addition, calculations were made (by means of standard dietary tables) of the average daily caloric and protein ingestion of each child. At Mooseheart the food consumption records were kept for seven consecutive days at the end of each 4-week period.

To determine the physical progress of the children throughout the study, body weights were taken every 2 weeks and height measurements were made once a month.

DISCUSSION OF RESULTS

Country Home results for cereal and wheat germ periods. The average grams of food consumed per day for each child at the Country Home have been summarized for each period in table 2. Of the seventeen children studied on all of the first three cereal periods, only four (J.M., G.L., E.Ku., and M.R.) showed a progressive increase in food intake as the cereal was changed from refined wheat to whole wheat to whole wheat plus 3.3 gm. stabilized wheat germ. The average grams of food intake for the three groups of children, however, for each of these three cereal periods were as follows:

Refined wheat	1591 gm. of food per child per day
Whole wheat	1598 gm. of food per child per day
Whole wheat + wheat germ	1607 gm. of food per child per day

It would thus seem that although the difference is small, the type of cereal may have an influence upon the child's appetite. Because the difference is small, these three periods have been grouped together as a control for further studies with the Country Home children.

The benefit of added amounts of wheat germ is more clearly brought out from a study of the results of a fourth period. It will be noted that when the wheat germ intake was increased to 20 gm. per day, the food consumption of every child without exception was definitely increased. The average food

TABLE 2

Average grams of food consumed per day, by each child, during each period

Group I. Country Home

PERIOD DATE	1. 9/16-11/10			2. 11/11-1/5			3. 1/6-2/2			4. 3/24- 4/20	5. 8/12- 9/8	6. 9/9- 10/6
Control period										Wheat germ (20)	Crystalline vitamin B	
	1 ¹	2 ²	3 ³	1 ¹	2 ²	3 ³	1 ¹	2 ²	3 ³			
C.J.			1243		1339					1681	1573	1522
J.A.	1584					1499		1208				
R.J.	1436					1429		1449				
L.T.					1617		1348				1933	1840
E.B.		1535		1526					1270			
J.M.		1332		1320					1425	1646		
H.G.		1487		1421					1476	1782	1760	
D.P.	1526									1965		
G.L.			1466		1458		1450			1708		
M.W.											1499	1441
G.P.		1630		1604					1551	2207		
Ed.P.			1740		1534		1686					
A.O.										1976	2192	2055
W.K.	1455					1343		1335		1980		
E.Ku			1759		1679		1662				2113	2036
M.R.			1555		1497		1458			2003		
G.W.										2064	2384	2340
J.T.											2168	1637
A.S.											1525	1395
M.L.	1835					1900		1958		2226		
J.S.											2197	2200
L.S.	1676					1764		1810		1967	2262	2302
T.O.	1495					1560		1630		1978	1949	1837
T.L.			2121		1923		1926			2261	2535	2518
J.B.											1969	1989
E.S.											1544	1896
E.Ka		1829		1873					1992			
D.H.											2154	2366
B.G.											2109	2139
C.C.										2309	2241	2128
L.L.										2055		
D.M.		2085		1931					1877	2301		
Ave.	1572	1650	1638	1613	1578	1583	1588	1565	1599		1972	1949
	1599									2003	1961	

¹ Refined wheat.² Whole wheat.³ Whole wheat + 3.3 gm. wheat germ.

intake for the entire group for this fourth period was 2003 gm. per day as compared to an average of 1599 gm. for the first three cereal periods.

TABLE 3

Average grams of food consumed per day by each child, during each period

Group II. Mooseheart

PERIOD DATE	1. 8/13-20	2. 9/9-15	3. 10/7-13	4. 11/4-11	5. 12/2-8	6. 12/30-1/5	7. 3/3-9	8. 3/31-4/6
DIET	No supplement			Stabilized wheat germ			Control	Crystalline vitamin B ¹
				<i>18 gm./day</i>				
B.A.	1679	1768	1874	2435	2248			2041
C.C.	1692	1863	1859	2002	1951		1731	2015
M.C.	1574	1812	1851	1973	1842	1973	2072	1903
M.D.	1757	1926	1963		1904	2036	1841	2097
F.D.	1755	1874	1874	2186	1993	2214	1774	2122
K.E.	1721	1881	1807	1987	1942	2198		2242
E.F.	1522	1535	1737	1814	1737		1642	1938
E.G.	1708	1889	1976	2408	2583		2112	2442
A.G.	1886		1976		2472	3143	2612	2558
I.M.	1671	1834	1845	2018	1896	2212	1870	2122
M.P.	1721	1941	1938	2283	2003	2015	2024	1981
A.P.	1622	1928	1757	2281	2160	2585	1870	2537
B.P.	1654	1843	1810					
I.W.				1724	1781	2002	1609	
M.R.	1807	1922	1921	2185	2138	2414	1882	2255
B.R.	1730	1838	1813	2033	1902	2187	1688	1979
B.W.	1600	1793	1816	2012	1940	2069	1909	1967
P.W.	1835	1715	1736	1988	1887	2078	1750	2125
D.W.	1730	1832	1853	1937	1791	1962	1798	1964
D.S.	1764	2017	1907	2278	2133	2636	1933	2083
G.S.	1566	1722	1752	1848	1805	1923	1767	1929
J.B.		1826	1802	1851	1883	2238	1892	2068
S.B.		1645	1704	1881	1804	2077	1570	
Ave.	1700	1829	1844	2056	1991	2220		
	1791			2089			1873	2116

¹ Four 0.1 mg. tablets 'Betaxin' per day.

Mooseheart results for control and wheat germ periods. The average grams of food consumed per day for the children at Mooseheart have been summarized in table 3. During the first 3 months the children consumed an average of 1791 gm. of food per day. During the following 3 months, while they

were receiving about 18 gm. of wheat germ daily, the average food consumption increased to 2089 gm. per day. During a subsequent control period the daily intake decreased to 1873 gm. The increase with these children was not quite so great as that observed with the Country Home children. The Mooseheart girls, however, were more normal subjects, and the greater increase at the Country Home might have been due to a previous retardation of these children before the convalescent period during which they were studied.

Results with crystalline vitamin B. In order to determine whether the vitamin B of the wheat germ may have been responsible for its influence upon the appetite, crystalline vitamin B in amounts comparable to the vitamin B content of the wheat germ was given to both groups of children. At the Country Home the average grams of food consumed per day during the two successive 4-week crystalline vitamin B periods were 1972 and 1949. Thus the stimulation of appetite with crystalline vitamin B gave results quite close in amount to the average intake of 2003 gm. which was taken by the children during the wheat germ period. At Mooseheart similar results were obtained. During the crystalline vitamin B period the children consumed an average of 2116 gm. of food, as compared to 1873 gm. during the 4-week control period immediately preceding the crystalline vitamin B period, and the averages of 2089 gm. for the wheat germ period and 1791 gm. for the first 3-month control period.

In order to determine the magnitude of change in vitamin B ingestion which can affect the child's response, the vitamin B contents of the diets and supplements have been determined by means of standardized biological assay technic as described by Schlutz and Knott ('36) with the improvement in technic as noted by Knott and Schlutz ('37). The vitamin B contents of the different cereals, the stabilized wheat germ, and aliquots of the total amount of food served on representative days are given in table 4. Since the quantity of second servings consumed increased the amount of food intake from 1.5 to 2 times above the original servings for which vitamin B values are

recorded in table 4, the children received from 260 to about 420 international standard units of vitamin B per day. A change from refined wheat to fortified whole wheat cereal increased the daily vitamin intake by 33 units or about 10% of the total daily intake. Apparently larger increases than this

TABLE 4

The vitamin B contents of diets and supplements in terms of international standard units of vitamin B

	ASSAY RESULTS (TOTAL DOSE/TOTAL GAIN)	FACTOR TO CONVERT TO I.S. UNITS	UNITS/GM.	UNITS/DAY
Cereals				
Refined wheat	1.57	1.49	0.43	6.5
Whole wheat	0.46	1.49	1.45	21.7
Whole wheat + 3.3 gm. wheat germ	0.30	1.49	2.22	40.0
Stabilized wheat germ	0.06	1.92	8.62	150-172
Country Home food				
10/ 2/35	0.68	1.49		277
10/24/35	0.82	1.49		229
11/18/35	1.01	1.49		183
12/19/35	0.99	1.49		183
1/ 9/36	0.96	1.49		205
2/ 7/36	1.68	1.49		108
4/13/36	2.13	1.49		98
4/17/36	1.71	1.49		128
				Ave. 176
Mooseheart food ¹				
3/3/37	1.90 gm.	1.92		63
3/4/37	0.78 gm.	1.92		224
3/5/37	0.79 gm.	1.92		112
3/6/37	0.87 gm.	1.92		138
3/7/37	0.95 gm.	1.92		112
3/8/37	1.13 gm.	1.92		101
3/9/37	0.84 gm.	1.92		153
				Ave. 129
Milk 3/4/37	5.9 cc.	1.92		84
Crystalline vitamin B	1.15 γ	1.92	452,500	200
Betaxin	1.9 γ	1.92	275,000	120
International standard absorbate	5.21 mg.	1.92	100	

¹ Total day's food, minus milk and bread, as weighed for sample servings.

are necessary before the level of vitamin B ingestion is able materially to affect appetite. During stabilized wheat germ and crystalline vitamin B periods, the vitamin B intake was increased about 50%. On the basis of the degree of stimulation obtained in this study, it would therefore seem that additional amounts of at least 150 units of vitamin B might be required to produce much effect upon appetite stimulation with young children.

A comparison of results on the basis of body surface area. Since the children in this study ranged in size from 15.7 to 45.8 kilograms, in order to compare results with individual children, the grams of food consumed per day have been calculated on a per kilogram basis. This treatment of the data, however, does not offer the best means of interpretation, since the amounts consumed per kilogram decrease with increases in weight. When the results are calculated on the basis of body surface area, a better comparison is possible between the younger and older children. The average grams of food per square meter of body surface for representative children of group I at the Country Home and group II at Mooseheart have been plotted in figure 1. From these charts it will be seen that the increases in vitamin B intake were able to produce increases in food consumption for nearly every child studied. There is more fluctuation in results between individual children, however, with the slightly older more normal girls observed at Mooseheart.

Relation of food consumption to weight gains. Since an increase in food consumption might be expected to produce gains in body weight, the changes in weight of the children have been analyzed. For this purpose the data for the Mooseheart girls have been presented (table 5) rather than that obtained at the Country Home, since it is believed that these girls were more capable of presenting normal responses. When results for the individual children were studied, however, it was observed that a direct relationship between grams of food consumed and gain in weight did not occur in all cases.

In order to explain this apparent lack of correlation between food consumption and weight gain, the caloric ingestion of group II is given in table 6, and certain data have been summarized for figure 2. It will be seen from these data that an increase in grams of food did not always mean an increase in calorie consumption. For example, the gram intake of the

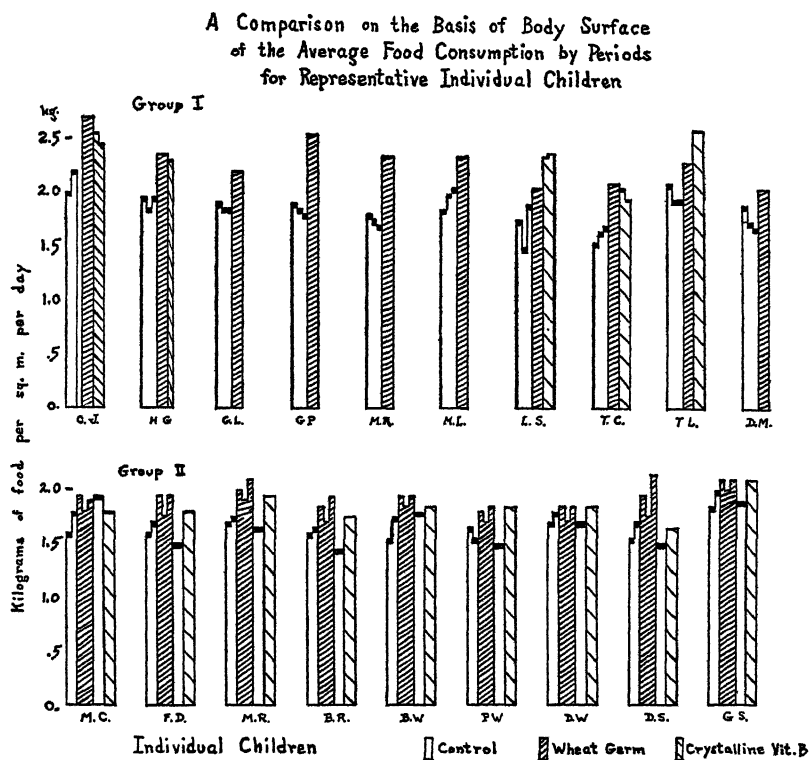


Figure 1

children during the January period was the highest of all periods, but the ratio of calories to grams in the food served was low for this period. Accordingly, the average calorie consumption was somewhat lower for this period, and weight gains were comparable to several other periods instead of being increased. The real effect of increased vitamin B intake

upon food consumption and weight is best seen in the November period. Here the grams served for first helpings and the caloric content of the food were nearly the same as the preceding period, but both the grams of food intake and the

TABLE 5
Weight changes of the girls in group II

	INITIAL WEIGHT	MONTHLY CHANGES CONTROL PERIOD			TOTAL GAIN FIRST 3 MO.	MONTHLY CHANGES WHEAT GERM PERIOD			TOTAL GAIN SECOND 3 MO.	GAIN FOR CRYSTAL-LINE VITAMIN B MONTH
	7/21	8/18 ¹	9/15	10/13		11/10	12/8	1/5		
	kg.									
B.A.	30.5	-1.1	+1.1	+0.5	0.5	+0.7	+0.8	+0.9	2.4	1.5
C.C.	25.8	-0.7	+0.7	+1.0	1.0	+0.3	+0.4	1.0
M.C.	28.1	-1.1	+1.2	+0.9	1.0	+0.4	+0.3	-0.1	0.6	0.7
M.D.	32.8	-0.8	+2.2	+0.1	1.5	2.5	0.3
K.E.	28.8	-1.1	+1.7	+0.1	0.7	+0.2	-0.2	+0.5	0.5	0.6
F.D.	32.1	-0.6	+1.1	+0.3	0.8	+1.1	-0.3	+1.3	2.1	0.1
E.F.	24.0	-0.9	+0.8	+0.6	0.5	+0.7	-0.6	+0.1	0.2	0.6
E.G.	40.0	-0.7	+2.0	+0.5	1.8	+1.5	+1.4	-1.5 ²	1.4	1.7
A.G.	36.6	-0.2	+1.4	-0.3	0.9	+2.0	4.3	1.1
I.M.	32.9	-1.1	+1.3	-0.6	-0.4	+0.4	-1.0	+1.3	0.7	0.8
M.P.	29.8	-0.7	+1.6	+0.1	1.0	+1.2	-0.1	+0.5	1.6	0.6
A.P.	38.7	-1.1	+1.4	+1.0	1.3	+1.1	1.8	1.4
B.P.	25.6	-0.1	+1.3	+0.1	1.3	
M.R.	29.9	-0.4	+1.6	+0.4	1.6	+1.1	+0.1	+0.9	2.1	0.6
B.R.	29.6	-0.7	+1.3	-0.3	0.3	+1.3	-0.2	+0.8	1.9	0.7
B.W.	26.7	-0.9	+1.6	+0.2	0.9	+0.4	+0.6	-0.1	0.9	0.0
P.W.	33.1	-1.0	+1.1	+0.2	0.3	+1.1	+0.5	+0.3	1.9	0.5
D.W.	26.9	0	+1.3	+0.6	1.9	+0.7	-0.1	-0.8	-0.2	0.3
Ave.		-0.7	+1.4	+0.3	0.9	+0.9	+0.3	+0.3	+1.5	+0.8
	8/18									
G.S.	21.5	+0.8	+1.1	1.9	+0.5	+0.6	-0.1	1.0	0.6
D.S.	30.9	+1.9	+3.0	4.9	+1.4	+1.0	+1.3	3.7	1.4
	9/15									
J.B.	43.6	+1.3	...	-0.5	+0.4	+1.1	1.0	2.2
S.B.	39.1	-1.7	...	+1.4	-0.2	+1.0	+2.2	..

¹ Low weights because of strenuous exercise.

² Upper respiratory infection.

calorie intake per square meter of body surface were definitely increased. During this month the average weight gain for the group was the highest of any 4-week period being 0.9 kg. or 2 pounds.

Other factors, however, than the actual calorie consumption of the girls must be considered. When ages were examined it was noted that there was a slight correlation between

TABLE 6

Average calories of food consumed per day, per square meter, by each child, during each period

Group II

PERIOD DATE	1. 8/13-20	2. 9/9-15	3. 10/7-13	4. 11/4-11	5. 12/2-8	6. 12/30-1/5	7. 3/3-9	8. 3/31-4/6
DIET	No supplement			Stabilized wheat germ			Control	Crystalline vitamin B
B.A.	1572	1441	1837	2186	1850	1747
C.C.	1517	1842	2235	2253	2203	2208	2138
M.C.	1659	1699	2165	2077	2022	1653	2410	1930
M.D.	1596	1704	2044	1826	1536	2042	1937
F.D.	1735	1718	1783	2138	1806	1915	2054	1868
K.E.	1639	1526	1909	1910	1864	1774	2108
E.F.	1759	1516	2061	1961	1987	2151	2130
E.G.	1393	1404	1828	1956	2053	1968	1936
A.G.	1610	1821	1814	2060	2243	1964
I.M.	1515	1459	1854	1875	1728	1819	1934	1919
M.P.	1690	1675	1908	2163	1873	1531	2147	1800
A.P.	1377	1338	1474	1794	1685	1721	1769	2036
B.P.	1813	1686	2053
I.W.	1867	1857	1703	1927	2015
M.R.	1758	1596	2090	2099	2034	1946	2058	2115
B.R.	1703	1510	1840	1923	1765	1667	1981	1713
B.W.	1717	1576	2025	2170	2087	1725	2248	2077
P.W.	1742	1483	1784	1943	1904	1804	2016	1988
D.W.	1867	1601	2120	1984	1892	1685	2209	1959
D.S.	1590	1656	1802	2079	1858	1823	1935	1766
G.S.	1967	1817	2385	2188	2216	1991	2449	2074
J.B.	1369	1541	1553	1573	1596	1674	1635
S.B.	1232	1452	1694	1594	1529	1581
Ave.	1661	1564	1910	1991	1886	1749		
	1712			1875			1953	1945

greater weight gains occurring with the older girls. It is believed that a few of these older girls may have been approaching the age of puberty. The length of stay at Mooseheart also had an effect in that the girl presenting the greatest gain had just joined the group. It is thought that she might have

undergone a period less favorable for growth immediately before her entrance to Mooseheart. A possible explanation of the low gains obtained throughout the study might be the protein ingestion of the girls. During all periods the grams of protein intake ranged from only 2.13 to 2.51 gm. per kilogram of body weight, with an average of 2.27 gm. The 2.51 gm. were consumed during the November period of increased

Average Gram and Calorie Values
for the different periods

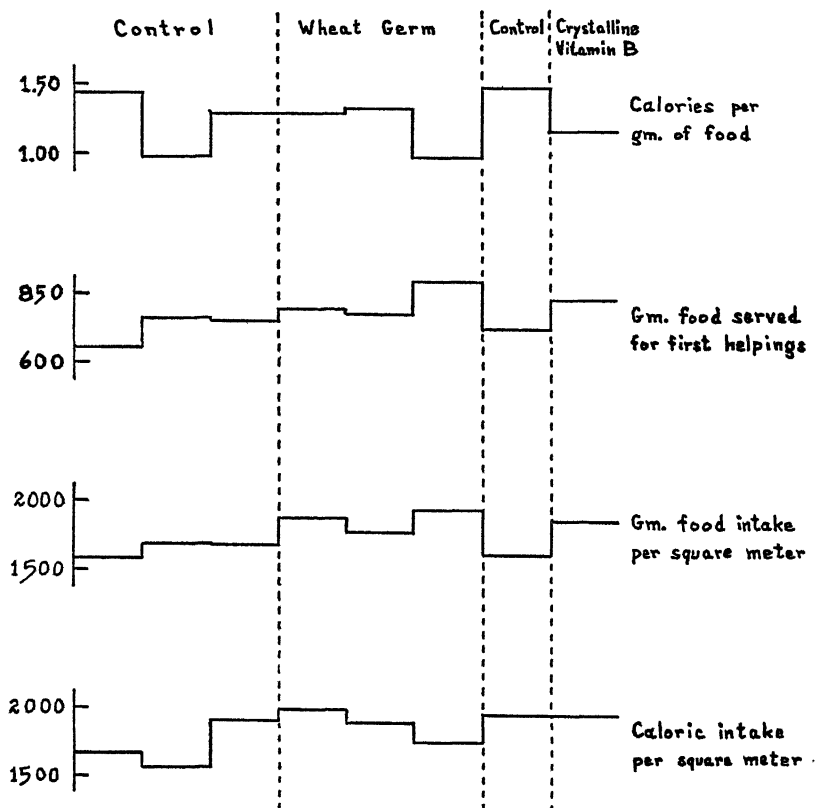


Figure 2

intake. Since Daniels et al. ('35) have presented data indicating that 3.2 gm. of protein per kilogram may be the optimum requirement, the low intake during the present study may be significant.

As a further check upon the results of the study, the complete market orders for group II for the first 6 months have been compared with market orders for a comparable group of girls for the preceding year. Examination of these data showed that the most significant difference between the two groups was the increased consumption of milk for group II during the 3 months they were receiving the stabilized wheat germ.

Other factors which may influence appetite. It is, of course, well known that appetite may be affected by many factors. To test some of these factors, during certain periods at the Country Home and at Mooseheart, records were kept of the primary choice of the children for cereals and of their desire for second servings.

In the primary selection of cereals, the girls at Mooseheart were allowed to vote on which of five cooked cereals they wished served. The results were as follows: malted wheat endosperm 110 votes, wheat endosperm 95, toasted whole wheat 74, oatmeal 47 and cracked wheat 45. It will be seen that in this primary selection of cooked cereals, the malted wheat cereal received 30% of the total votes.

When the results in regard to second servings were analyzed, it was observed that the least quantity of cereal was consumed during the period immediately after a time of cereal restriction. Thus, established habits would appear to be important in controlling appetite. The average results for the 4617 breakfasts which are included in the records indicated that in addition to habit such factors as flavor, consistency, and psychic appeal were important. A decided preference was expressed for the prepared cereals. Of the cooked cereals, those with more flavor or containing more of the whole grain were consumed with greater readiness. It is interesting to note that

of the refined cooked cereals, again the one with malted additions to the wheat endosperm was selected with most frequency.

SUMMARY

Factors affecting appetite have been studied through food consumption records which have been kept during 32 weeks for thirty-two children, age 4 to 10, at the Country Home for Convalescent Crippled Children, and during a similar period for twenty-two girls, age 9 to 11, at the Mooseheart Home for Child Training. Following control periods, the vitamin B intake of both groups was increased by means of: first, stabilized wheat germ, and second, crystalline vitamin B.

The vitamin B contents of the regular diets of the children averaged from 260 to 420 international standard units per day, while the supplements of wheat germ and crystalline vitamin B furnished from 120 to 200 additional units. A supplementary ingestion of approximately 150 units of vitamin B (representing an increase of about 50% in the daily vitamin B intake) produced increases of from 17 to 25% in the grams of food consumed per child per day.

The caloric ingestion during these periods of increased food consumption correlated to a slight degree with increased weight gains.

CONCLUSIONS

Since the higher levels of vitamin B administered during this investigation produced no apparent ill effects, did not force the growth, and did tend to stabilize the appetites of the children, it is concluded that the higher ingestions of vitamin B may be regarded as optimum.

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STUDIES ON THE VITAMIN B₁ REQUIREMENTS OF GROWING RATS ^{1, 2}

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TWO FIGURES

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The encouraging results we have obtained with chicks fed an autoclaved natural grain ration supplemented with graded levels of crystalline vitamin B₁ (Arnold and Elvehjem, '38) led us to try to adapt the ration for similar studies with rats. Several trials indicated that the autoclaved grain ration and the purified rat rations generally used were somewhat low in factor W (Elvehjem, Koehn and Oleson, '36; Frost and Elvehjem, '37). Since preparations containing this factor but deficient in vitamin B₁ were available at this laboratory, studies on a vitamin B₁ deficient purified rat ration were initiated. The results obtained indicate that it is possible to obtain normal rates of growth in rats when a suitably fortified vitamin B₁ deficient synthetic ration is supplemented with crystalline vitamin B₁ hydrochloride.

EXPERIMENTAL

Albino rats with an initial weight of 40 to 50 gm. were placed on experiment at about 25 days of age. The cages were equipped with raised wire screen bottoms (two meshes to the inch). Food and water were each supplied daily in glazed

¹ Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

² Presented in part at the ninety-fourth meeting of the American Chemical Society at Rochester, September, 1937.

³ Wilson and Company Fellow.

porcelain cups. Scattering of feed was minimized by the use of suitable glass covers on each cup. The covers contained openings of $\frac{1}{2}$ inch diameter which permitted ready access to the ration. Food consumption was recorded daily. The animals were weighed weekly.

To determine whether the autoclaved natural grain ration which has proved to be successful in the chick assay procedure could be used with equal success in rat assays, the following studies were made.

Ration 240A developed by Kline, Keenan, Elvehjem and Hart ('32) served as the basal ration for the rats. It has the following composition:

Autoclaved portion:	
Ground yellow corn	59
Pure flour middlings	25
Crude domestic acid precipitated casein	12
Untreated portion:	
CaCO ₃ (precipitated)	1
Ca ₃ (PO ₄) ₂ (precipitated)	1
Iodized salt (0.02% potassium iodide)	1
Cod liver oil	1

The rats fed the unsupplemented basal ration (fig. 1, group 47) gained in weight for 2 or 3 weeks and then remained practically constant in weight for a number of weeks. The food intake of the animals was low and after 4 to 6 weeks on the ration the animals exhibited a vitamin B₄ deficiency. The backs of the animals were hunched, the jowls protruded and the animals walked high on their rear legs with ataxia. The symptoms of polyneuritis appeared 2 to 4 weeks later. These results have been discussed elsewhere (Elvehjem and Arnold, '36). Control groups which received supplements of 2.0 (group 48), 2.5 (group 49), or 3.0 (group 50) % of standard yeast 3 (10 I.U. vitamin B₁ per gram) grew moderately well during the 6-week experimental period.

To induce a greater food intake by the animals in the early stages of the experiment, the ration was modified to contain greater amounts of other nutritive factors. Four per cent

autoclaved yeast⁴ was added as a source of the heat stable vitamin B complex. Ten per cent hulled peanuts was added since this material supplies considerable vitamin B₄ (Kline, Bird, Elvehjem and Hart, '36). Additional amounts of unsaturated fatty acids were also supplied by this product. In

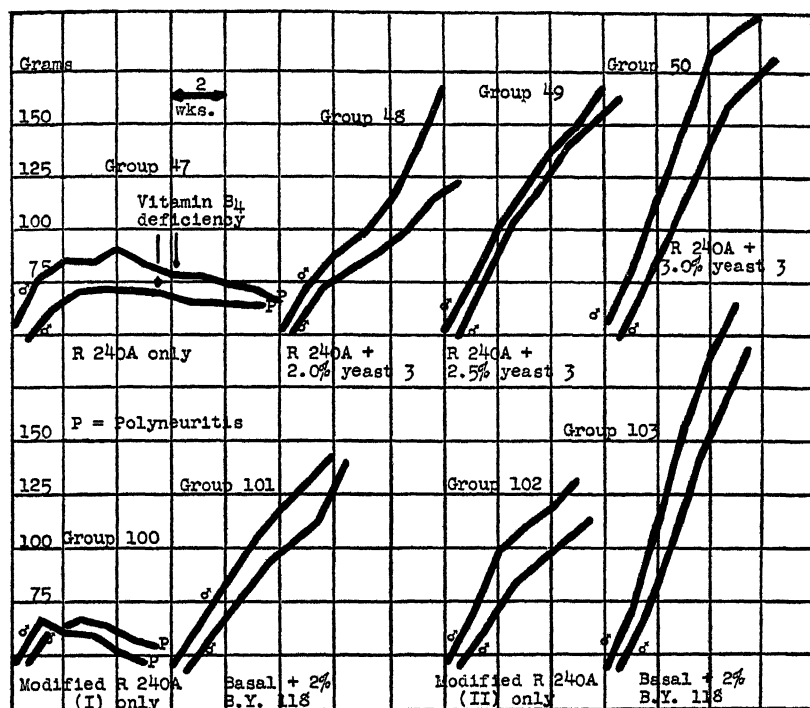


Fig. 1 Individual growth records of rats fed ration 240A and modifications of the autoclaved grain ration.

addition to the above supplements the effect of the addition of certain animal tissues was also tested. In one case autoclaved lung was added and the flour middlings were omitted in order to keep the protein level of the ration constant. The modified ration had the following composition.

⁴ Strain C, Anheuser-Busch, St. Louis.

Modified ration 240A (I)

Autoclaved portion:	
Ground yellow corn	70
Crude domestic acid precipitated casein	12
Hulled peanuts	5
Yeast ⁵	4
Vacuum de-aerated lung ⁶	5
Untreated portion:	
Iodized salt	1
CaCO ₃ (precipitated)	1
Ca ₃ (PO ₄) ₂ (precipitated)	1
Cod liver oil	1

Rats fed this ration exhibited the typical vitamin B₁ deficiency during the fifth week (fig. 1, group 100). Positive control group 101 (fig. 1) received 2% brewers' yeast 118 in addition to the basal ration. The growth of the animals in this group cannot be considered normal even though an adequate supply of vitamin B₁ was present in the ration.

Results obtained by Elvehjem, Koehn and Oleson ('36) indicated that liver extract contained liberal amounts of a factor which possessed growth stimulating properties for rats. Two per cent liver extract, therefore, was substituted for the 4% autoclaved yeast in the ration.

The growth of the rats fed this ration (modified R 240A (II)) supplemented with 2% brewers' yeast 118 (fig. 1, group 103) was excellent. This result indicates clearly that liver extract supplied those factors which were deficient in the original basal. However, the rats fed the unsupplemented basal ration continued to grow slowly. The liver extract, therefore, added sufficient vitamin B₁ to protect the rats from polyneuritis and even permitted some growth to occur.

Since the autoclaved grain ration did not appear to be suitable for vitamin B₁ studies with rats unless considerably modified, a new ration was developed. This ration (ration 112) has the following composition:

⁵ See footnote 4, page 431.

⁶ Wilson Laboratories, Chicago.

Sucrose	62
Purified casein	18
Autoclaved peanuts	10
Autoclaved yeast ⁷	10
Salts I	4
Factor W	≅ 2 liver extract
Halibut liver oil for vitamins A and D	2 drops twice weekly

The casein was prepared from diluted skim milk by precipitation with dilute hydrochloric acid, and further purified by solution in dilute ammonium hydroxide, and precipitation with dilute acid. This process was repeated three times. Hulled fresh peanuts have been included in the ration as a source of vitamin B₄ and unsaturated fatty acids. The peanuts were ground and autoclaved for 5 hours at 15 pounds pressure before being used. Autoclaved yeast⁷ was used to supply the heat stable components of the vitamin B complex. Salts I (Phillips and Hart, '35; Kline, Bird, Elvehjem and Hart, '36) was used throughout.

The growth stimulating principle of liver extract has been designated factor W by Frost and Elvehjem ('37). The following procedure was used for the preparation of this fraction free of vitamin B₁: 100 gm. of liver extract were dissolved in 200 cc. of water and 1 liter of ethyl alcohol and 1200 cc. of ethyl ether were added with constant stirring. The precipitate was allowed to settle out and the supernatant liquid siphoned off. The precipitate was dissolved in 100 cc. of water and reprecipitated. After being washed with an ether-alcohol-water mixture, the precipitate was dissolved in 400 cc. of water and allowed to stand for 24 hours, whereupon a light colored precipitate settled out, which was centrifuged off and washed with 25 cc. of water. The combined filtrate and washings were concentrated in vacuo at 50°C. to 80 cc. Upon the addition of 10 volumes of acetone, a dark gummy precipitate formed. The precipitate was dissolved in 50 cc. of water and reprecipitated with 10 volumes of acetone. After being washed with acetone, the precipitate

⁷ See footnote 4, page 431.

was dissolved in 250 cc. of water and allowed to stand, whereupon more insoluble material settled out, which was centrifuged off and washed with 25 cc. of water. The combined filtrate and washings were diluted to 300 cc. with alcohol and stored at low temperature for use.

The procedure used in making up the rations was to dry the water solution of factor W on the casein in the drying room (50°C.) before a fan. Supplements to be added to the ration which were also contained in solution, such as vitamin concentrates, were then dried in vacuo over calcium chloride on weighed portions of the dry purified casein-factor W component of the diet. The sucrose, autoclaved yeast and autoclaved peanuts were combined in the proper proportions and ground in a Burr mill before use.

The results obtained when the rats were placed on the basal alone or on the basal plus vitamin B₁ supplements at weaning are given in table 1. Rats fed the unsupplemented basal ration showed the polyneuritic syndrome in about 5 to 6 weeks. Supplements of 0.3% brewers' yeast 131,⁸ 0.2% international standard or 60 micrograms of crystalline vitamin B₁ hydrochloride⁹ (natural source) per 100 gm. of ration 112 resulted in average growth rates of 3.0 to 3.2 gm. per day for the 6-week experimental period. A lower growth rate resulted when rats were fed 50 micrograms of vitamin B₁, obtained as a concentrate from natural sources, per 100 gm. of ration 112. The average daily gain of these rats was 2.8 gm. for the 6-week period. An increase of the same vitamin B₁ supplement to 100 micrograms per 100 gm. of ration 112 resulted in a much improved rate of gain amounting to approximately 3.8 gm. daily. The responses in growth of the rats when they were fed ration 112 supplemented with sources of vitamin B₁ such as brewers' yeast 131, the international standard or as the vitamin itself show that ration

⁸We are indebted to Dr. Harold Levine, of the Premier-Pabst Corporation, Milwaukee, for several generous samples of yeast.

⁹The crystalline vitamin B₁ hydrochloride samples were kindly weighed out by Mr. H. A. Campbell on a micro-analytical balance.

TABLE 1

Growth of rats fed ration 112 alone or ration 112 supplemented with vitamin B₁ from brewers' yeast 131, the international standard adsorbate or vitamin B₁ preparations for a 6-week experimental period

SUPPLEMENT TO RATION 112	RAT NUMBER	INITIAL WEIGHT	WEIGHT 6 WEEKS	DAILY GAIN
None		gm.	Polyneuritis	
	102-21	37	20 days	
	102-22	58	28	
	102-23	56	24	
	104-71	50	46	
	104-72	50	46	
	108-61	52	46	
	108-62	49	55	
	118-90	39	41	
	118-00	43	47	
Average	9 ♂	48	39	
0.3% brewers' yeast 131	108-41	49	gm. 202	gm. 3.2
	108-42	54	174	
	109-21	50	191	
	109-22	49	155	
	118-85	41	185	
	Average	5 ♂	49	
0.2% international standard	109-01	48	165	
	109-02	48	173	
	118-73	37	170	
Average	3 ♂	44	169	3.0
0.00006% crystalline vitamin B ₁ (natural source)	118-05	38	160	
	118-95	40	178	
Average	2 ♂	39	169	3.1
0.00005% vitamin B ₁ (natural source)	106-61	42	168	
	106-62	42	168	
	108-71	52	163	
	108-72	47	166	
	109-81	49	136	
	109-82	49	170	
Average	6 ♂	47	165	2.8
0.00010% vitamin B ₁ (natural source)	108-81	50	192	
	108-82	44	215	
	108-83	44	210	
Average	3 ♂	46	206	3.8

112 is practically complete in all nutrient essentials for rats aside from vitamin B₁.

To obtain greater differences between the groups receiving graded levels of vitamin B₁, the animals were depleted of the major part of their vitamin B₁ reserves before they were given their supplements. The results given in table 2 indicate that rats restricted to the basal ration for a 10- to 14-day depletion period required a higher level of vitamin B₁ in the ration to make growth gains comparable to those reported in table 1. Extensive comparisons are not possible since only the international standard is common to both tabulations. It is interesting to note, however, that the ratio of the international standard to vitamin B₁ is approximately the same in both tables. Comparable growth gains resulted when the rats were fed ration 112 supplemented with 0.2% international standard or 60 micrograms of crystalline vitamin B₁ (natural source) per 100 gm. of ration (table 1). Approximately equal growth responses resulted when the rats were fed ration 112 supplemented with 0.2% international standard or 60 micrograms of synthetic crystalline vitamin B₁ hydrochloride (sample 2) per 100 gm. of ration (table 2). Another comparison may be made. The growth responses which resulted when the rats were fed ration 112 supplemented with 0.25% brewers' yeast 132 or 60 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration (table 2) are approximately the same. An increase of one-third in each of these vitamin B₁ sources resulted in equal growth responses. The average daily gains which resulted when the rats were fed ration 112 supplemented with 0.33% brewers' yeast 132 or 80 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration are also comparable. When the rats were fed 100 micrograms of vitamin B₁ (tables 1 and 2) comparable growth gains resulted which indicated that this amount of vitamin B₁ in the ration was close to the optimum value.

In view of the good rates of growth reported here it is interesting to examine the vitamin B₁ intake of the animals that were fed ration 112 supplemented with crystalline vitamin B₁ hydrochloride. The values were derived from weekly

TABLE 2

Growth of rats fed the basal ration for a 10- to 14-day depletion period, then fed ration 112 alone or ration 112 supplemented with vitamin B₁ from brewers' yeast 132, the international standard adsorbate or vitamin B₁ hydrochloride (sample 2) for a 5-week experimental period

SUPPLEMENT TO RATION 112	RAT NUMBER	INITIAL WEIGHT	WEIGHT 5 WEEKS	DAILY GAIN
None		gm.	Polyneuritis	
	120-11	33	34 days	
	120-21	61	51	
	123-72	51	35	
	123-81	52	35	
	126-03	62	56	
	126-06	61	47	
	126-12	66	56	
	127-62	74	34	
	127-71	71	28	
Average	9 ♂	59	42	
0.25% brewers' yeast 132	120-33	68	gm. 106	gm.
	126-05	64	150	
	126-25	66	172	
	126-33	58	158	
	127-86	56	144	
Average	5 ♂	62	146	2.4
0.33% brewers' yeast 132	126-14	62	160	
	126-26	64	152	
	126-32	58	206	
Average	3 ♂	61	173	3.2
0.2% international standard	123-65	64	125	
	123-94	66	165	
	126-01	66	200	
	126-15	65	125	
	126-16	74	215	
	127-61	84	170	
	127-74	81	182	
	127-87	62	188	
Average	8 ♂	70	165	2.7
0.00006% vitamin B ₁ (sample 2)	120-12	55	129	
	122-54	56	104	
	126-11	70	186	
	126-31	65	190	
	127-73	82	168	
Average	5 ♂	66	155	2.5
0.00008% vitamin B ₁ (sample 2)	123-91	61	166	
	127-63	94	205	
	127-75	88	204	
Average	3 ♂	81	192	3.2
0.00010% vitamin B ₁ (sample 2)	126-21	69	186	
	126-23	62	176	
	126-24	66	215	
Average	3 ♂	66	192	3.6

food consumption records. The data charted in figure 2 give a comparison of the average daily intakes of vitamin B₁ hydrochloride of the animals that received 60, 80 or 100 micrograms of crystalline vitamin B₁ hydrochloride (sample 2) per 100 gm. of ration 112. The average daily consumption progressively increased from 3.8 to 5.7 micrograms of vitamin B₁ hydrochloride when the ration contained the lowest level

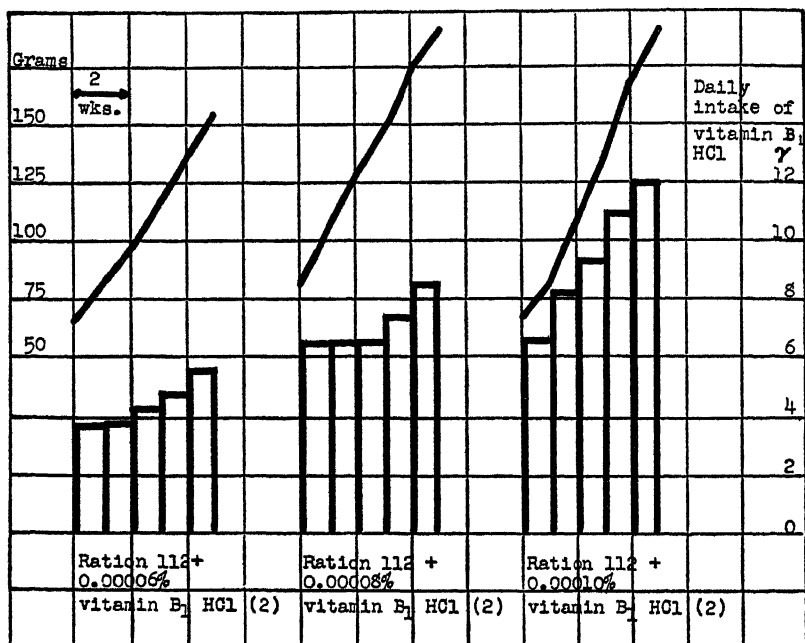


Fig. 2 Average daily intake of the vitamin B₁ hydrochloride of rats fed ration 112 supplemented with 60, 80 or 100 micrograms of vitamin B₁ hydrochloride (sample 2) per 100 gm. of ration.

of vitamin B₁, from 6.6 to 8.6 micrograms of vitamin B₁ hydrochloride when it contained the intermediate level and from 6.7 to 12.7 micrograms of vitamin B₁ hydrochloride when it carried the highest level of vitamin B₁.

The figures obtained from food consumption records may also be used to determine the vitamin B₁ ingested per kilogram body weight per day. The values were calculated from

the records of the rats which received 100 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration 112 and are calculated on the basis of the average weights of the animals each week as determined by the weights at the beginning and end of the 7-day period. The results given in table 3 are expressed in terms of the crystalline vitamin B₁ hydrochloride and its international unit equivalency (3 micrograms of vitamin B₁ hydrochloride (sample 2) \cong 1 international unit) on the basis of previous data (Arnold and Elvehjem, '38).¹⁰

TABLE 3

Daily intake of vitamin B₁ hydrochloride (sample 2) expressed in terms of body weight. Record obtained from rats that received 100 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration 112

WEEK	MEAN WEIGHT	FOOD CONSUMPTION TOTAL FOR WEEK	VITAMIN B ₁ · HCl INGESTED PER DAY	VITAMIN B ₁ · HCl INGESTED PER KILOGRAM BODY WEIGHT PER DAY	
	gm.	gm.	micrograms	micrograms	international units
1	74	47	6.7	91	30
2	94	58	8.3	88	29
3	122	66	9.4	77	26
4	152	76	11.0	72	24
5	180	85	12.1	68	23

DISCUSSION

The results submitted here demonstrate quite clearly that while symptoms of vitamin B₁ deficiency can be produced in rats on a variety of vitamin B₁ low rations, normal growth can be obtained through the addition of pure preparations of the vitamin only when the basal ration contains an adequate supply of the other essential factors. Certain difficulties may be encountered even in the production of uncomplicated polyneuritis if the basal ration is too low in certain essential

¹⁰ Several different samples of the crystalline synthetic vitamin have been used and some variation in potency has been observed. However, in our preliminary runs no attempt was made to check the moisture content of the crystals before use. Our purpose was to study the effect of the addition of the crystalline material to the basal ration rather than the variation in potency of individual samples. Studies on the comparison of the potency of different samples are now in progress.

factors and contains appreciable amounts of vitamin B₁. In this case a prolonged feeding period is required and other deficiencies develop before the animal is completely depleted of the anti-neuritic factor.

Although it is difficult to set a definite figure for the normal growth rate of rats, a growth approaching 4.0 gm. per day must be considered very good. Very recently Edgar and Macrae ('37) obtained a daily growth of 4.3 to 4.6 gm. over a 4- to 6-week period for male rats on a purified ration supplemented by the daily administration of 50 micrograms of riboflavin, 10 to 20 micrograms of vitamin B₁ hydrochloride, fullers' earth filtrate \cong 1 gm. of yeast and fullers' earth eluate \cong 2 gm. yeast. The properties of the fullers' earth eluate factor agree in many points with those for vitamin B₆ (György, '35 a, b; Harris, '35; Birch and György, '36; Lepkovsky, Jukes and Krause, '36) and the fullers' earth filtrate factor with those for factor W (Elvehjem, Koehn and Oleson, '36, and Frost and Elvehjem, '37) and the filtrate factor of Lepkovsky and Jukes ('36). The high levels used by Edgar and Macrae probably compensated for any destruction of the above factors which may have taken place during the autoclaving of the yeast extract which they used for the preparation of their concentrates.

The lack of a good source of factor W was probably the reason for the reduced growth rates observed by Bender and Supplee ('37). These investigators supplied 10 micrograms of riboflavin, 100 mg. of autoclaved rice polishings concentrate and 12.5 micrograms vitamin B₁ hydrochloride daily to rats on a purified diet. The average daily growth rate was approximately 1.25 gm. over a 42-day period. When the riboflavin supplement was increased to 20 micrograms daily, the average growth of the rats was approximately 1.7 gm. per day during a 42-day period. These investigators suggested that their basal ration might not supply sufficient amounts of other factors not well differentiated at that time. Waterman and Ammerman ('35) observed an average daily growth rate (males) of approximately 2.2 gm. when fed the basal ration

supplemented with 10 micrograms of vitamin B₁ hydrochloride daily. When the vitamin B₁ hydrochloride supplement was increased to 20 micrograms daily the average daily growth (males) was approximately 2.9 gm. over a 45-day period. Since the latter investigators used 15 parts of autoclaved yeast in the diet, it appears that this source cannot always be depended upon to supply adequate amounts of factor W.

The results submitted here indicate that the addition of an adequate amount of factor W to a vitamin B₁ deficient basal ration reduced the vitamin B₁ requirements of the rats. The animals fed 60 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration 112 obtained an average of 3.8 micrograms of vitamin B₁ hydrochloride daily during the first week of the experiment. The daily food consumption increased during the course of the experimental period so that during the last week on experiment the rats ingested an average of 5.7 micrograms of vitamin B₁ hydrochloride daily. The average growth for this group was 2.5 gm. per day. Similarly the vitamin B₁ intake of the rats fed 80 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration 112 rose from an average of 6.6 to 8.6 micrograms of vitamin B₁ hydrochloride daily during the course of the 5-week experimental period which resulted in an average growth rate of 3.2 gm. per day for the period. The vitamin B₁ ingested by the rats fed 100 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration 112 increased from an average of 6.7 micrograms of vitamin B₁ hydrochloride daily to 12.1 micrograms daily during the experimental period. The average daily growth for this group was 3.6 gm. per day. Calculations based on food consumption records show that the vitamin B₁ hydrochloride ingested by the rats in the last group decreased from 30 international units of 23 international units per kilogram of body weight per day during the course of the experimental period. It is impossible to establish the exact optimum vitamin B₁ intake per kilogram of body weight for rats since the experiments have not been continued for extended periods of time.

Although the results submitted here are encouraging we do not propose that the rat growth method is sufficiently developed for assay purposes. The data yield definite information regarding the vitamin B₁ requirements for normal growth. Ration 112 appears to contain ample amounts of essential nutritive factors other than vitamin B₁. The procedure to be used for assay purposes, however, must depend on more extensive collaborative observations.

SUMMARY

1. The autoclaved grain ration (ration 240A) cannot be used for studies on vitamin B₁ with rats unless it is considerably modified.

2. A synthetic vitamin B₁ low ration (ration 112) has been developed which includes several recently disclosed factors of the vitamin B complex.

3. Rats restricted to ration 112 supplemented with 80 to 100 micrograms of vitamin B₁ hydrochloride per 100 gm. of ration grow at a normal rate.

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THE RELATION OF THE 'GRASS JUICE FACTOR' TO GUINEA PIG NUTRITION¹

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FIVE FIGURES

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Earlier work in this laboratory (Elvehjem, Hart, Jackson and Weckel, '34) demonstrated a seasonal variation in the nutritive value of mineralized milks. Rats fed a mineralized milk, produced by cows on good pasture, grew at a rate of 4 to 4.5 gm. per day during a 6-week experimental period. When milk, produced by cows receiving a winter barn ration, was used as the basal diet, growth of only 2 to 2.5 gm. per day was obtained. Later it was demonstrated (Kohler, Elvehjem and Hart, '36) that the addition of fresh lawn clippings or the pressed juice from grass to the mineralized winter milk produced a rate of growth comparable to that obtained on summer milk.

In a more recent publication (Kohler, Elvehjem and Hart, '37) results were presented to show that various supplements rich in the known vitamins produced little or no growth response in rats on a basal winter milk diet. This led to the conclusion that the growth stimulating factor of grass was distinct from all the known vitamins. At this time it was pointed out that considerable variation was encountered in different groups of rats when placed on the basal ration, presumably due to differences in the storage of certain factors in the young rats when started on the experiment. Since

¹ Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

guinea pigs are herbivorous, we felt that this species might be a more suitable animal for the assay of a factor which is found most abundantly in fresh green plant tissue.

The present paper deals with the growth of guinea pigs on mineralized winter milk and the growth-promoting action of supplements similar to those used in our earlier work with rats.

EXPERIMENTAL

Two preliminary experiments were made in order to study the reaction of guinea pigs to diets made up largely of whole milk. The milk used was obtained fresh each morning from the same cow (Holstein) at the dairy barn. The whole milk was fed twice daily in sufficient quantities to allow ad libitum consumption. In addition to the milk each guinea pig received a daily mineral supplement consisting of 1 mg. of Fe as ferric pyrophosphate, 0.1 mg. of Cu as copper sulfate, and 0.1 mg. Mn as manganese sulfate. The salts were incorporated into a small amount of dextrin and fed in a small gelatin capsule. The animals were weighed daily.

In the first trial four guinea pigs weighing about 300 gm. each were used. Two pigs were placed on the basal mineralized milk alone, one on the basal plus 2 cc. of orange juice daily and one on the basal plus 5 gm. of fresh oat grass which had been grown in the greenhouse.

For the second experiment six guinea pigs were divided into three groups of two pigs each. Two pigs received the basal mineralized milk diet and the four remaining pigs were given the same diet except that the milk was aerated for 1 hour at 60 to 65°C. Two of the pigs receiving the aerated milk were given 1 mg. of ascorbic acid per animal daily. The aerated milk was used to determine if a variation in the vitamin C intake would alter the results. Growth curves for the ten pigs are given in figure 1.

The sequence of events observed when the pigs were placed on the milk diets was very similar in each animal. During the first week or two the animals lost considerable weight. After

this preliminary period, they apparently became accustomed to the liquid diet and consumed fairly large amounts of milk. For the next few weeks they remained at constant weight or in some cases gained slightly until their original losses were regained. Between the fourth and seventh weeks the animals began to lose weight rapidly, some losing as much as 70 to 80 gm. in 1 week. At this stage respiratory trouble was noted in most cases. Immediately preceding death, clonic contractions of the legs were observed.

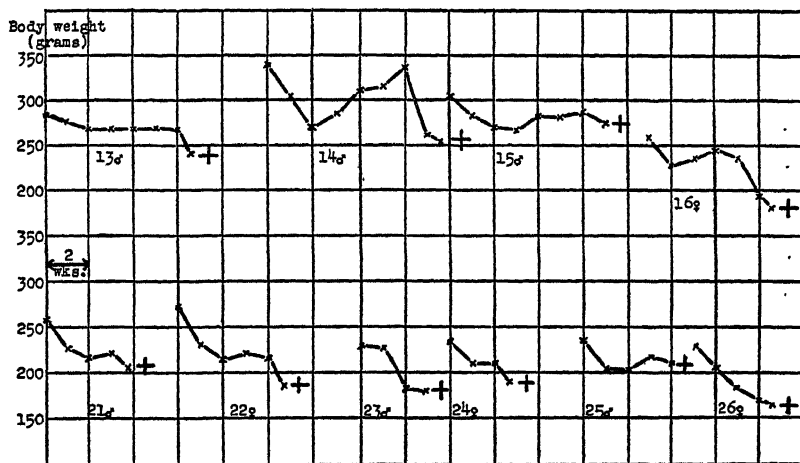


Fig. 1 Weight records of guinea pigs receiving mineralized winter milk (ad lib.). Nos. 13, 14, 25 and 26 received whole raw milk. No. 15 received raw milk + 5 gm. greenhouse oat grass daily. No. 16 received raw milk + 2 cc. orange juice daily. Nos. 21 and 22 received aerated milk. Nos. 23 and 24 received aerated milk + 1 mg. pure vitamin C daily.

Autopsies were made on all the animals and no signs of scurvy or rickets could be detected. In many cases the lungs showed advanced states of inflammation and congestion and, in some animals, necrotic areas in the lungs were evident. Since all animals showed a temporary favorable response on the milk after the first week or two, it appears that the animals actually suffered from a dietary deficiency which brought on the extreme debility and susceptibility to infection.

Since the addition of orange juice or oat grass did not alter the changes observed, it is obvious that neither vitamin C nor vitamin P (Armentano et al. '36) were limiting factors. The oat grass was tested on rats simultaneously and found to be inactive as a source of the 'grass juice factor.' Thus the failure of this oat grass to stimulate growth in guinea pigs did not eliminate the importance of this factor in the nutrition of guinea pigs.

The animals receiving the aerated milk died a little sooner than the other pigs. However, no signs of scurvy were evident even in these animals. The addition of generous amounts of ascorbic acid gave no better results than those observed in the pigs on the untreated milk. The uniformity of the results obtained in the preliminary trials indicates that winter milk is deficient in one or more essential factors necessary for the normal development of guinea pigs.

Various supplements were fed to guinea pigs receiving a basal diet of winter milk plus iron, copper and manganese in an attempt to correct the deficiency observed. In all of the following experiments the salt solutions together with the supplement were placed in a clean dish with a little milk each morning. Later in the day, when the animal had completely consumed the contents of the dish, enough milk was added to insure ad libitum feeding. Since milk contains 2 to 2.5 mg. of vitamin C per 100 cc., and the guinea pigs consumed 80 to 200 cc. of milk per day, there should be no danger of an inadequate supply of this vitamin. Unpublished work in our laboratory has shown that 0.3 mg. of pure ascorbic acid per day is sufficient to prevent the onset of scurvy and to support a good rate of growth. However, as a precautionary measure all animals were given $\frac{1}{2}$ cc. of orange juice daily by pipette.

In figure 2 are presented growth curves of guinea pigs which received a series of supplements chosen to supply ample quantities of various known vitamins. The supplements were as follows: 5 gm. whole wheat meal, 5 gm. white cornmeal, 2 gm. brewers' yeast, 1 gm. vacuum dried whole

liver (pork),² 0.5 gm. liver extract powder,² and 0.5 gm. 92% alcohol soluble liver extract.² The wheat and cornmeal were found to be ineffective at the original levels, but when 7.5 gm. per day were fed, fair rates of growth were obtained. The vacuum dried whole liver gave some growth when the level was increased to 1.5 gm. per day, but neither of the liver extracts was effective. Apparently, the active principle of the liver is not extracted, or is destroyed by the commercial

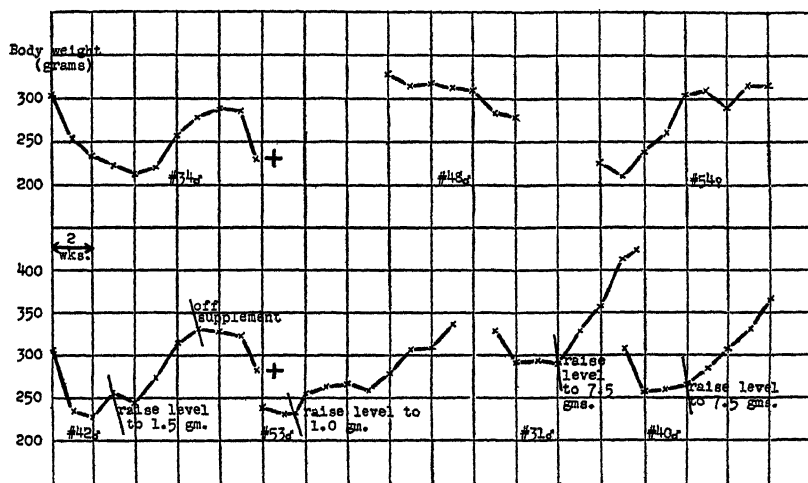


Fig. 2 Weight records of guinea pigs receiving mineralized winter milk (ad lib.) plus supplements containing liberal quantities of known vitamins. The daily supplements were as follows: No. 34, control; no. 48, 0.5 gm. 92% alcohol-soluble liver extract; no. 54, 2.0 gm. brewers' yeast; no. 42, 1.0 gm. vacuum dried whole liver; no. 53, 0.5 gm. liver extract powder; no. 31, 5.0 gm. whole wheat meal; no. 40, 5.0 gm. white cornmeal. All received 0.5 cc. orange juice daily.

processes used in the preparation of liver extracts. The inferior response given by the yeast together with the remarkable growth produced by grasses, shown in figure 3, adds credence to the hypothesis that the 'grass juice factor' described for rats is the same as the limiting factor concerned here.

² We are indebted to Dr. David Klein, Wilson Laboratories, Chicago, for the samples of liver and liver extract used.

The effects of feeding dried grasses as supplements to guinea pigs on the mineralized winter milk are shown in figure 3. The three cereal grasses (nos. 462, 458, 455)³ were produced under comparable conditions. They were grown at the same time on plots of the same field so that they were exposed to identical weather conditions. They were cut at the same stage of growth, 1 month after emergence, and were dried at 82°C. for 4 hours.

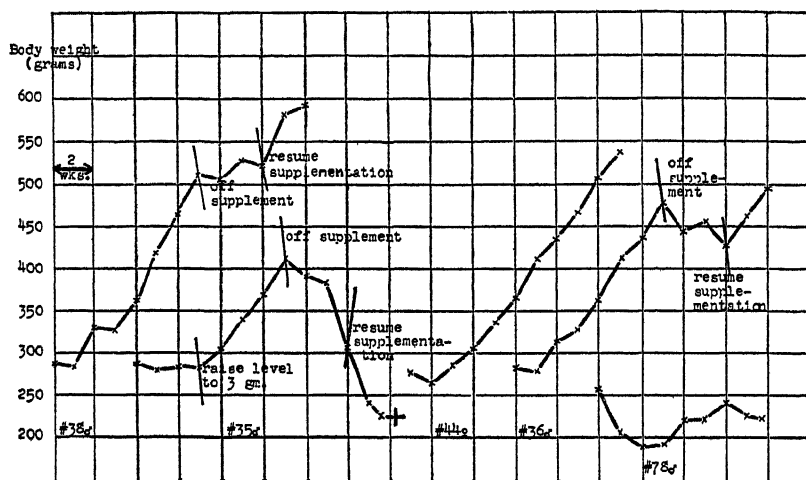


Fig. 3 Weight records of guinea pigs receiving mineralized winter milk (ad lib.) plus dehydrated grasses. The daily supplements were as follows: no. 38, 2.0 gm. barley grass no. 462; no. 35, 2.0 gm. oat grass no. 455; no. 44, 2.0 gm. barley grass no. 462; no. 36, 2.0 gm. wheat grass no. 458; no. 78, control. All received 0.5 cc. orange juice daily.

The barley grass, which was the most effective, produced a growth rate of 5.3 gm. per day from the second to the seventh week of the experiment. The wheat grass was only slightly less potent than the barley grass. However, in the case of the oat grass, it was necessary to raise the level to 3 gm. per day to produce good growth.

³ These grass samples were generously supplied by Mr. C. F. Schnabel of the American Butter Company.

After 7 weeks on experiment, pigs nos. 35, 36 and 38 were taken off the supplement and fed the mineralized milk alone. Growth stopped almost immediately. After 3 weeks on the basal ration alone, during which period these animals actually lost weight, supplementation was resumed. Once again remarkable growth resulted in the animals receiving the barley and wheat grasses (pigs nos. 36 and 38). The animal receiving the oat grass (pig no. 35) was in such poor condition

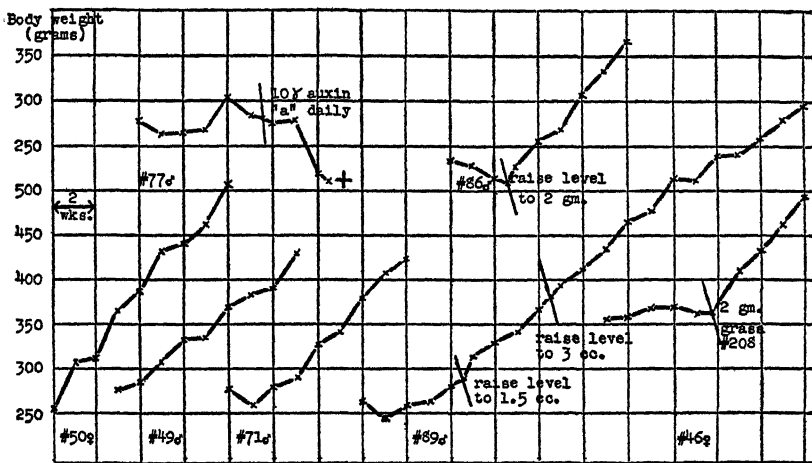


Fig. 4 Weight records of guinea pigs receiving mineralized winter milk (ad lib.) plus various preparations of grass or auxin. The daily supplements were as follows: no. 50, 20 gm. fresh lawn clippings; no. 49, 20 cc. juice pressed from lawn clippings; no. 71, 1.0 gm. frozen oat grass pulp no. 207 (batch 1); no. 89, 0.75 cc. juice of oat grass pulp no. 207; no. 46, control for 4½ weeks, 2.0 gm. dehydrated oat grass no. 208 (Arnold drier); no. 77, control for 5½ weeks, then 10 gamma auxin 'a'; no. 86, 1.0 gm. frozen oat grass pulp no. 207 (batch 2). All received 0.5 cc. orange juice daily.

at this time that it refused to eat the supplement when it was offered. This animal died about 2 weeks later. These results show definitely that the grasses contain a nutritional factor which is essential for maintenance as well as growth of guinea pigs.

The growth curves in figure 4 show that guinea pigs do not need roughage in their diet, at least when milk is used as the

basal ration. One guinea pig received 20 gm. of fresh lawn clippings (mostly Kentucky blue grass) per day as the supplement, while another received 20 cc. of the centrifuged press juice from fresh lawn clippings. Twenty cubic centimeters of this juice contained about 0.2 gm. solids. Both the clippings and the juice were quite effective, although neither was as good as barley grass no. 462, which was fed at a comparable level. Pulped oat grass no. 207⁴ which had been frozen immediately after cutting and pulping was tested and found to be more potent than the freshly cut lawn clippings. Two batches of this pulp were obtained. Of the first batch, 1 gm. was sufficient to promote good growth. The second batch was less potent, 2 gm. per day being required. Since this pulp contained only about 25% solids, the effective levels were 0.25 to 0.50 gm. per day on the dry basis. The centrifuged press juice from this pulp was also effective; 1.5 to 3 cc. per day, containing 8% of solids or 0.12 to 0.24 gm. dry matter, gave definite growth stimulation. A portion of the grass used to prepare pulp no. 207 was dried in an Arnold drier⁵ and designated as grass no. 208.⁶ This dehydrated oat grass was fed to a guinea pig on the winter milk basal ration, and it was found that about 2.0 gm. daily were necessary to produce good growth. Hence in the dehydration process some destruction occurred.

Since green plant tissues which are growing most rapidly are the most potent sources of the factor in question, it was thought that it might be worthwhile to test the plant hormone, auxin. Hence, auxin 'a'⁷ was fed at a level of 10 gamma per day to a guinea pig on the winter milk basal diet. The growth

⁴ See footnote 3, page 450.

⁵ In the Arnold drier (also called Heil drier and made by the Heil Company), the initial temperature was 1400°F. and the final temperature 250°F. The total time of drying the grasses was 3 minutes. During this period, evaporation proceeds at such a rate that the actual temperature of the grass is considerably less than that of the surrounding gas. Natural gas was used as fuel. The combustion gases are drawn through the drying drum, and give a partial CO₂ atmosphere.

⁶ See footnote 3, page 450.

⁷ Kindly furnished us by Dr. F. Kögl, Utrecht, Holland.

curve is also given in figure 4. Although no response was obtained, the possibility that auxin is the factor is not excluded since the level fed may have been too low. Our supply was not great enough to feed other pigs at higher levels.

Some idea as to the stability and solubility of the active principle of the grasses was obtained in later experiments, the results of which are given in figure 5. The effect of temperature during storage of grass is shown by the growth of the animals receiving grasses no. 75 and no. 76.⁸ These samples were portions of the same batch of dehydrated rye grass, which were stored under different conditions for a period of 6 months. Grass no. 75 was kept in a cooler at a freezing temperature, while grass no. 76 was kept at room temperature which at one time rose as high as 95°F. It is evident that considerable loss occurred during storage at room temperature, while the sample stored in the cold retained its activity.

Lot no. 73,⁸ a mixture of wheat and rye grasses, was divided into two parts. One-half of the mixture was fed to a guinea pig as a supplement to mineralized winter milk, while the other half was autoclaved for 1 hour at a pressure of 15 pounds per square inch, dried in the drying room (65°C. for 24 hours), and tested on another guinea pig. The weight records indicate that much of the activity was destroyed by this treatment.

Three samples of dried alfalfa were tested, two of which had been produced under ordinary farm conditions as contrasted to the third, sample no. 380,⁸ which had been cut at the stage of most rapid growth and dried in an Arnold drier.⁹ The latter sample was quite potent, while the field-dried samples were very poor in growth promoting activity. This difference cannot be ascribed entirely to the method of drying, since the samples were not cut at comparable stages of growth, and further the inactive samples had been stored at ordinary barn temperatures, while sample no. 380 was kept in the

⁸ See footnote 3, page 450.

⁹ See footnote 5, page 452.

cooler until the feeding tests were made. Also the samples were grown on different soils in different sections of the country.

Other growth curves given in figure 5 show that the factor is not extracted from dehydrated barley grass (no. 462) by

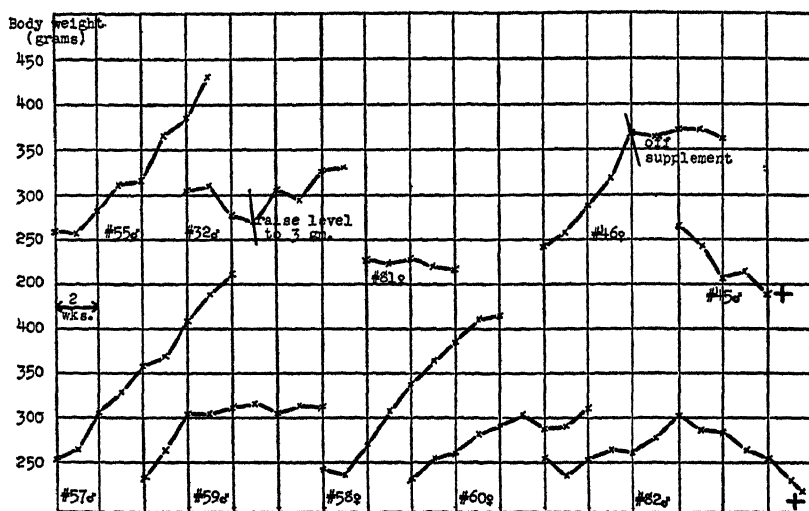


Fig. 5 Weight records of guinea pigs showing the stability of the grass juice factor to heat and its solubility in petroleum ether. The daily supplements were as follows: no. 55, 2.0 gm. dehydrated alfalfa no. 380 (Arnold drier); no. 32, 2.0 gm. alfalfa hay, sample 1; no. 81, 2.0 gm. alfalfa hay, sample 2; no. 46, residue from petroleum ether extraction of grass no. 462; no. 45, petroleum ether extract of grass no. 462; no. 57, 2.0 gm. dehydrated rye grass no. 75 (stored in cooler for 6 months); no. 59, 2.0 gm. dehydrated rye grass no. 76 (stored at room temperature for 6 months); no. 58, 2.0 gm. wheat and rye grass no. 73; no. 60, 2.0 gm. autoclaved grass no. 73; no. 82, control. All received 0.5 cc. orange juice daily.

petroleum ether (Soxhlet extraction for 24 hours). The fractions were fed at levels corresponding to 2 gm. per day of the original grass. The petroleum ether extract, which constituted about 5% of the original grass, was inactive, while the residue retained most of the potency.

DISCUSSION

Although at present there is no indisputable proof that the rat and guinea pig factors are the same, the following considerations indicate that this is the case.

The same basal ration, winter milk supplemented with minerals, has been used to produce the deficiency in both species.

Sources of the potent factor (or factors) are parallel in activity for the two species. Grass samples which were effective for one species have proved to be potent for the other, and, conversely, grass samples which have been inactive for one have also proved to be inactive for the other species. Further, yeast showed inferior activity for both species.

The limited data available at present on loss of activity of grasses upon heat treatment, drying, and storage are parallel for the two species.

The pressed-out juice of potent grasses contains the active factor for both rats and guinea pigs.

Up to the present we have not been able to obtain good growth in guinea pigs receiving mineralized whole milk produced by cows on pasture (Summer, '37). This can probably be attributed to the fact that the pastures were in a very poor condition due to a sustained dry spell. Further, the guinea pig's requirement for the factor is apparently much greater than that of the rat, so that a 'summer milk' which would support good growth in the rat might not be good enough to support growth in the guinea pig. Further work will be done with 'summer milk' when better pastures are available.

In this connection it is interesting that Riddell et al. ('36), in studies designed to show the effects of winter and summer rations of cows on the vitamin C content of the milk, report that, although no appreciable difference was noticeable in incidence of scurvy, 40 cc. of pasture milk fed as a supplement to the basal scorbutic diet produced much better growth in guinea pigs than did a similar supplement of milk produced by cows on a dry ration. Cows receiving silage produced a

milk of intermediate growth promoting qualities. It is probable that these differences were associated with the growth factor with which we have been working.

In view of the indications pointed out above we have assumed, as a working hypothesis, that the rat and guinea pig factors are the same, and further studies are being carried out on stability, chemical properties, and concentration using the latter species for assay.

As was pointed out in our previous paper, the combination of mineralized winter milk, orange juice and yeast supplies in adequate quantities all of the essential minerals, protein and energy, as well as the following known vitamins, C, P, A, B₁, B₆, nicotinic acid amide (anti-blacktongue factor), factor W, flavin, choline and the chick antipellagra factor. Further, unpublished data from this laboratory have shown that several grasses which are rich in the 'grass juice factor,' are very poor sources of the chick pellagra factor, vitamin B₄, the chick gizzard factor, and the highly unsaturated essential fatty acids. That vitamin D was not involved was shown in earlier work by feeding cod liver oil to rats on mineralized winter milk. It is on the basis of the above facts that we postulate the existence in grasses, and less abundantly in other food materials, of a new essential factor in nutrition.

An interesting fact brought out by this work is that guinea pigs can be raised on an all liquid diet in spite of the fact that their digestive tract is equipped to handle large amounts of roughage. Thus, the animals receiving mineralized milk, orange juice and grass juice grew at a good rate and no abnormalities were observed. When the grass juice was omitted the animals died. In contrast to guinea pigs, rats on a mineralized winter milk diet do not die, but grow continuously, although at a slow rate.

Several earlier workers (Bartenstein, '05; Moro, '07) attempted to raise guinea pigs and rabbits on all milk diets, and were uniformly unsuccessful. Their work, however, was done before it was appreciated that milk is low in iron, copper and manganese, so that deficiencies of these elements may have complicated the picture.

Several workers have attempted to raise calves on a milk diet supplemented with iron and copper. Bennet ('32) and Herman ('36) reported that, even with the addition of Fe, Cu, and Mn, calves will not live more than 8 to 13 months on an exclusive milk diet. Herman stated that although the calves grew better than normal on the mineralized milk diet for the first 6 to 8 months, they began to show signs of debility and lost weight after this period. All the animals died by the thirteenth month except one which lived to the seventeenth month. It would be interesting to repeat his experiment adding supplements of a potent grass juice to his ration. By such a method it might be possible to raise a calf on a liquid diet.

Duncan, Huffman and Robinson ('35) attribute the failure of complete nutrition in calves, limited to a milk diet, to a low blood magnesium.

Virtanen ('36) and Virtanen and Lane, ('36 a, '36 b) have published a series of papers showing that various green plant constituents reach maximum concentrations when plant growth is most rapid. Thus, protein, tryptophane, aspartic acid, carotene, and vitamin C are present in the largest quantities just before the plant reaches the flowering stage.

Hunt, Record and Bethke ('36) have shown similar variations in the vitamin B₁ and flavin content of pasture grasses and hays which were correlated with the rate of growth of the plants.

We would like to emphasize the fact that the potency of grasses in the growth stimulating factor with which we have been working also seems to vary in a similar manner with the stage of growth, the mature plants being much less effective than rapidly growing ones. Ordinarily, farmers allow their hay crops to reach a mature stage of growth before harvesting and at this stage the growth-stimulating activity is relatively low. This fact together with destruction of the active principle during drying and storage accounts for the seasonal variation in milk which has been discussed in an earlier paper. This seasonal variation led to the discovery

of the grass juice factor which later work has shown to be distinct from other known nutritional essentials.

SUMMARY

1. Winter milk supplemented with iron, copper, and manganese is an inadequate diet for young guinea pigs. In contrast to rats, which grow slowly on mineralized winter milk, guinea pigs die on such a diet.

2. Orange juice, brewers' yeast, and liver extract produce little or no beneficial effect when fed as supplements to this diet.

3. Various grasses contain a factor (or factors) which is essential for maintenance and growth of guinea pigs. Small supplements of such grasses enable guinea pigs on a mineralized winter milk diet to grow normally.

4. The active principle of grasses is soluble in the plant juices since centrifuged grass press juice is active.

5. The activity of grasses disappears upon storage at room temperature. It is fairly stable at lower temperatures. It is destroyed to a large extent by autoclaving.

6. From experience with the guinea pig it is probable that this species can be used to good advantage in further studies on the 'grass juice factor.'

We are indebted to Mr. S. B. Randle for help in carrying out some of the more recent work reported in this paper.

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THE TECHNIC OF MEASURING RADIATION AND CONVECTION ¹

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ONE FIGURE

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Heat is the most important end product of the chemical reactions within the body but relatively little attention has been paid to the mechanism of heat loss. Vaporization which accounts for one-quarter of the loss has been studied in detail. Only a few attempts have been made to separate radiation from convection and the word convection is hardly mentioned in physiological literature. Until fairly recent times the words conduction and Leitung were employed lumping together the two factors to which physicists apply the terms conduction and convection.

The thermal radiation from the human body is normally the largest factor of the heat loss. The amount of energy thus lost may be calculated from the formula of Stefan and Boltzmann. This fundamental equation which contains all the factors upon which the radiation loss depends is:

$$Q, k \cdot e \cdot e' \cdot S_0 (T_b^4 - T_o^4).$$

Q, number of calories radiated by the surface.

k, proportionality factor depending upon the effective radiating surface area (the profile area according to Lambert's law).

e, emissivity of the receiver (the 'blackness' of the surroundings from the radiation standpoint).

e', emitting power of the emitter ('blackness' of surface of the body).

S₀, Stefan-Boltzmann constant.

T_b, absolute temperature of the emitter (temperature of the surface of the body in °C. + 273).

T_o, absolute temperature of the absorber.

¹ Clinical Calorimetry no. 49.

Convection accounts for about 15% of the heat loss when a man is quiet but for much more than this if there be any considerable movement of air over the surface of the body. The term includes heat removed from the surface by currents of air or water. Attempts to formulate laws for convection have not been attended with any degree of success and most measurements are made by indirect methods. The factors involved under ordinary conditions are:

T_b , the temperature of the surface of the body.

T_o , the temperature of the air.

A , the area of the body.

V_a , the velocity or movement of the air with respect to the body surface.

K , proportionality factor which contains the specific heats of the convecting molecules, diffusion coefficients, etc.

Vaporization which accounts for about 25% of the total heat loss has been studied by many observers and a bibliography of this subject will be found elsewhere (Du Bois, '36). The factors involved are:

T_b , the temperature of the surface of the body.

T_o , the temperature of the air.

H , humidity of the air.

V_a , movement of the air with respect to the moist surface.

A , the moist area of the body.

S , the activity of the sweat glands depending on exercise, room temperature, emotion, etc.

D , the water content of the tissues, a factor of doubtful importance.

Conduction seldom plays an important role unless the body is in contact with a large and highly conducting surface. The amount of heat lost or gained depends on the temperature of the body and of the solid, the area and nature of contact and the specific conductivity of the solid and of the body tissues.

The warming or cooling of ingested food and drink is a factor that can be readily calculated. The loss of heat in urine and feces is taken care of in the calculation for warming the ingesta. The warming of air in the respiratory passages is included under the term convection; the loss of heat through the almost but not complete saturation of air in the nose and air passages is included under vaporization.

REVIEW OF LITERATURE

Rubner (1896) published figures regarding the partition of heat loss from a clothed man under one set of conditions but did not describe his methods, which could not at that time have been accurate. He had a full realization of most of the important factors and he very properly calculated the effective radiating surface as being less than the total surface. However, in his classic book ('02) he does not pay any attention to the division of losses between radiation and convection. Many others devised radiometers which were probably inaccurate. Aldrich made an accurate but rather cumbersome instrument ('22) and published ('28) some valuable data on radiation from the human body. Bohnenkamp ('32) and his co-workers aroused new interest in the subject and emphasized the importance of the effective radiating surface or profile surface. Bedford ('35 a, b) made good use of a radiometer. The most important recent discussion of technics will be found in the papers of Winslow, Herrington and Gagge ('36 a, b).

The respiration calorimeter of the Russell Sage Institute of Pathology (Lusk, '15) which has been used almost continuously since 1914, determines the total heat production by the method of indirect calorimetry. It also measures the total heat loss by the direct or physical method. Vaporization is measured by absorbing the water vapor in bottles of sulfuric acid which are weighed at the end of each period. All the heat of radiation plus convection is caught and measured by the stream of cold water that flows in the pipes near the top of the calorimeter. The accuracy is tested at frequent intervals by alcohol and electric checks. An idea of the operating precision during the last few years may be had from a consideration of the alcohol checks shown in table 1.

Attempts were made to devise a method of measuring convection directly but without success. It therefore became necessary to determine it by difference and this could be accomplished only if radiation were measured with accuracy. There being no satisfactory radiometer available at the time, Hardy ('34) devised one which proved rapid and accurate.

Since the characteristics of radiation vary greatly with wave length it was necessary to make spectrographic studies of the human radiation and its transmission through various media. For this purpose an infra-red spectrograph was devised by Hardy and will be described in another publication. Studies were next made by Hardy and Muschenheim ('36) which confirmed the generally accepted views that radiation from the human skin (temperature 20 to 37°C.) is all given off in the infra-red region with wave lengths between 5μ to 20μ with a maximum at about 9μ . This is far below the range of the sun's radiation since practically none of its waves longer than 2μ reach the earth's surface. In this region the human skin is within 1 or 2% of being a 'perfect black body radiator.'

TABLE 1
Alcohol checks made during experimental seasons

<i>Date</i>	<i>Heat</i>	<i>Error, per cent¹</i> <i>O₂</i>	<i>CO₂</i>	<i>Average R.Q.</i>
February 12, 1935	+ 1.6	— 0.4	+ 0.4	0.661
November 14, 1935	— 0.9	+ 1.4	+ 0.8	0.666
May 15, 1936	+ 1.4	— 0.9	+ 1.8	0.666
February 2, 1937	— 0.2	— 1.0	— 0.8	0.667
March 11, 1937	+ 1.6	— 1.3	— 1.7	0.664

¹ Oxidation of 10 cc. of solution = 52.4 Cals., 14.14 gm. CO₂, 15.42 gm. O₂ and H₂O, 9.39 gm.

It absorbs practically all of this infra-red radiation, reflects none and transmits none. Layers of epidermis 0.1 mm. in thickness absorb 95% of these radiant heat waves and transmit the resulting heat by conduction and convection. Hardy and Muschenheim ('36) found no significant difference between the white skin and the deeply pigmented Negro skin in this range of wave length which is so far below the visible spectrum. Since white skin is practically a perfect black body, radiation depends directly on the skin temperature and the radiation technic, as shown by Hardy ('34), is the simplest method which will give skin temperatures with accuracy. The Hardy radiometer consists of eight blackened tinfoil receiving elements made up of eight bismuth alloy thermocouples and eight compensating thermocouples. Radiation of heat from the area of 10 sq.cm. at which the instrument 'looks' is concentrated by

means of a silver cone so that it all falls on the blackened disc. Calibration is made by means of a Leslie Cube into one side of which is let a blackened cone which is, for practical purposes, a perfect black body radiator. All calibrations are made at the temperatures of the surfaces to be tested and therefore at identical wave lengths. Readings which require about 10 seconds can be made on scales which show skin temperature and also radiation rate in small calories per square centimeter per second.

MEASUREMENT OF RADIATION

An exact determination of the total radiation from the human body requires many measurements and calculations. In our calorimeter experiments the naked man lies on a bed made of fish line with a mesh of about 4 cm. Under his hips and back is placed a single cotton sheet folded so as to give the necessary protection to the skin with as small an exposed surface as possible. Between the fish net head rest and the back of the head is a small folded hand towel. Conduction through the fish lines is so small that it can be neglected. The calorimeter which is just large enough to contain bed and man comfortably is a closed circuit apparatus with copper walls, a double plate glass window 75×70 cm. at the head and a small triple glass window 30×30 cm. on the right side. Glass is impermeable to radiation between 3.5μ and 20μ . The effective radiation temperature of the copper walls and bottom and the glass window which is measured at the same time as the radiation of the body, is always within one-tenth of a degree of the temperature of the air in the calorimeter. The copper coils at the top of the box are usually 3.5°C . cooler.

The average radiating temperature of the surface of the body is determined by pointing the radiometer at the twenty points on the surface shown in figure 1. These measurements are made by the subject himself just before and just after each basal hour and require only 2 to 3 minutes. The radiation values are read and recorded by an observer who watches the subject through the side window. The total surface of the body is estimated from the Sage height-weight, and linear

formulas (Du Bois and Du Bois, '16). The proportions of surface contributed by head, trunk, arms, legs, hands and feet are calculated from the linear formula and the average radiation per square centimeter from each of these is then multiplied by the percentage of the total area contributed by that part of the body.

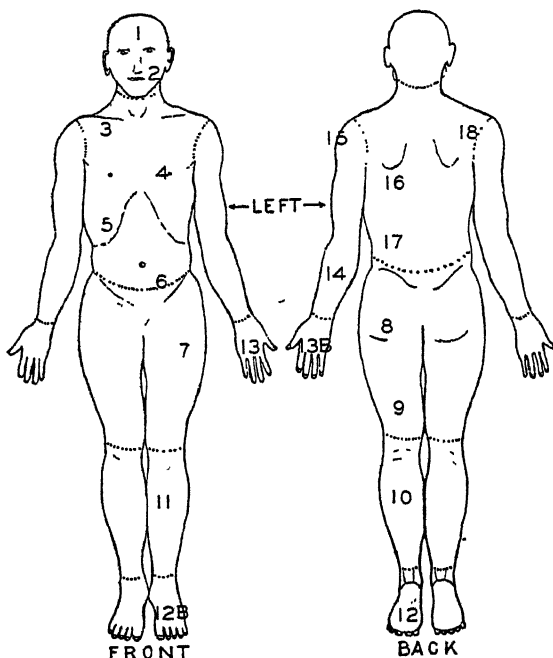


Fig. 1 Locations of individual areas over the body surface which were tested and the divisions of the skin surface for weighting.

From the above data the average skin temperature of the body may be calculated as follows:

$$T_s = (T_1 \times 0.07 + T_2 \times 0.14 + T_3 \times 0.05 + T_4 \times 0.07 + T_5 \times 0.13 + T_6 \times 0.19 + T_7 \times 0.35) \text{ where } T_s = \text{average skin temperature}$$

T_1 = head temperature	0.07 = weight for head surface
T_2 = arms temperature	0.14 = weight for arms surface
T_3 = hands temperature	0.05 = weight for hands surface
T_4 = feet temperature	0.07 = weight for foot surface
T_5 = legs temperature	0.13 = weight for leg surface
T_6 = thighs temperature	0.19 = weight for thighs surface
T_7 = trunk temperature	0.35 = weight for trunk surface

The various weighting factors were computed from the linear formula measurements of sixteen subjects.

The radiation temperature of the internal calorimeter surfaces is measured so that the radiation exchange between the subject and the calorimeter may be made. Thus

$$Q = 1.37 \times 10^{-11} (T_s^4 - T_c^4) \times t \times A \times f$$

Q = kg. Cals. per period lost to the calorimeter from the skin

T_s = average skin absolute temperature

T_c = average calorimeter absolute temperature

t = length of the period in seconds

A = total area in square meters

f = ratio of the effective radiating area to the total area = 0.78

The relationship of effective or profile radiating surface to the total surface area depends on the position of the body and extremities. We have adopted the standard position of lying flat on the back, legs close together and arms close to the body. Bohnenkamp and Ernst ('31) using a spread eagle position estimated the profile surface as 85% of the surface according to the linear formula. Badow and Bohnenkamp ('35) using the electrical capacity method in improved form find an average of 81% for men and 86% for women.

Bedford ('35) who studied men in the crouching position estimated 50%. Winslow, Herrington and Gagge, who made observations on two men, seated in a chair, obtained percentages of 74 and 75, values lower than those of Bohnenkamp as might be expected from the differences in position. We have made measurements on two normal men, one E.F.D.B., the subject of many experiments, and the other V.T., a man with short legs but exactly the same sized trunk as E.F.D.B. They were placed in the position of Egyptian mummies and wrapped in gummed paper according to the method of Du Bois and Du Bois ('16). Their 'mummy' surfaces were 78.3 and 78.4%, respectively, of their total surfaces. For purposes of calculation we have adopted for the present the standard figure of 78%, realizing that there may be an error as great as 2 to 3%.

The radiation exchange may be precisely determined by measuring the projection area of each part of the body surface and the radiation exchange of this surface with the part of the environment to which it is exposed. These values are integrated over a hemisphere and then added. Such a procedure is described in detail by Bohnenkamp and Pasquay ('31), although their numerical values are not correct. Such an experiment was performed on E. F. D. B. The radiation loss, using the photographically determined effective area, was 46.8 Cals. per hour and that measured by the weighting method just described was 45.8 Cals. per hour. The 2% difference is considered to be within the estimated experimental error of $\pm 3\%$.

CONVECTION

The stream of cold water which flows through the cooling pipes near the roof of the calorimeter removes all the heat of convection and radiation together. The temperature of the water is measured as it enters and as it leaves the box and the water that has flowed through the box is weighed at the end of each period. When the heat of radiation is subtracted from the calories found by this direct determination it leaves the heat lost by convection. The factor of conduction through the netting is too small to be significant.

In our experiments there was no ingestion of food or drink. When urine was voided into a wide necked bottle it was weighed on a Chatillon balance inside the calorimeter and its heat loss measured by thermometry or calculated from known rates of heat loss determined in many experiments. From a thermal standpoint the urine is considered as a separate body and its heat loss subtracted from the total to find the heat loss of the human subject.

The air flow through the calorimeter is adequate to remove the body gases but not sufficient to affect heat loss. The air enters the head of the calorimeter and is baffled so that there is no movement of air perceptible to the naked subject or to a delicate anemometer. The average humidity inside the box

is determined from the water 'residuals' (the water vapor content of 10 liters of calorimeter air which is tested at the end of each period). The humidity is usually low, ranging from 22% to 47%, the ordinary level being about 30%.

VAPORIZATION

The current of air leaving the calorimeter is passed through a blower and then through two tall glass flasks which contain concentrated sulfuric acid. These are weighed at the end of each period giving the water vaporized after corrections are made for changes in the moisture content of the air as determined by gravimetric analysis of 10 L samples of air at the end of each period. Care is taken to adjust the ventilation of the box so that there is little or no change in the humidity. This reduces to a minimum the error caused by loss or gain of water by the copper walls, bed or sheet. No experiment is started until the subject has been lying on the sheet at least an hour and sealed in the calorimeter $\frac{3}{4}$ hour. No experiments are attempted on days when the external humidity is high, as the walls on such days lose several grams of water in the early part of the experiment.

In calculating the heat lost in vaporization we employ the standard figure of 0.583 Calories for each gram of water which evaporates at the average calorimeter temperature of 25°C. It may be pointed out that to expand the saturated vapor to 25% relative humidity and 25°C. (room conditions) requires an additional 0.062 Calorie per gram which may be considered as part of the vaporization loss as it depends essentially on the number of grams of moisture evaporated. In this paper we have left this small fraction which amounts to 2 to 3% of the total heat loss in the Calories ascribed to convection. Perhaps it deserves a separate heading.

STORAGE OF HEAT

In some experimental procedures such as that described by Winslow, Herrington and Gagge ('36 a, b) the storage of heat in the human body is an important factor in the calculations.

In our procedure it is measured directly by means of the difference between the heat production and the heat eliminated. In addition the temperature of the body is estimated with an electrical resistance thermometer inserted 15 cm. in the rectum and read to 0.01°C . every 4 minutes, and by the twenty surface temperature readings taken at the beginning and the end of every period. Even with all these precautions the measurement is inexact because of differences in the rate of temperature change in various organs of the body and differences in the specific heats of these organs. This problem has been studied by the Sage investigators for the last 24 years. Coleman and Du Bois ('15) found evidence that with rapid changes of body temperature thermometers strapped on the surface gave better indications of average body change than rectal thermometers. Barr and Du Bois ('18) devised a method of determining average body change by direct calorimetry from the total heat produced as measured by indirect calorimetry. Burton ('35) using a similar calculation has found best agreement when the rectal temperature change is weighted as 65% and the average surface temperature as 35%. In our preliminary calculations of our own basal experiments we obtain best agreement by weighting the rectal temperature as 80% and the average skin temperature as 20%. This does not hold except in basal or near basal conditions. Gagge ('36) working with normal men finds that the lowering rectal temperature changes alone may be incorrect as indices of storage when the change is large but that rising rectal temperature changes associated with warm conditions ordinarily yield correct results. This, however, is certainly not the case in chills and fever or exercise as shown in a long series of fever studies made in the Sage calorimeter.

Fortunately our calculations of convection and radiation do not involve consideration of this uncertain factor of heat storage in the body. Our basis of calculation is total heat loss from the surface of the body and this is determined directly by physical methods, direct calorimetry. Of course simultaneous measurements of total heat production are made by the

method of indirect calorimetry using O_2 and CO_2 . The storage of heat in or loss of heat from the body is calculated also and an estimation of heat loss is furnished from the algebraic sum of total heat production and heat storage. In large series of experiments the totals of direct and indirect calorimetry agree closely. With individual periods of 1 hour the divergence in normal people may be $\pm 8\%$.

EXPERIMENTAL ROUTINE

The day before an experiment the room thermostat is adjusted to the desired temperature. The temperature of the room can be held to $\pm 0.5^\circ C$. and the periodicity of the fluctuations is about $\frac{1}{2}$ hour. The calorimeter room is provided with both cooling and heating units so that any desired temperature may be easily obtained. At 8 A.M. of the morning of an experiment the water is started circulating through the cooling coils of the calorimeter and the temperature of the box is maintained by a heating unit in the calorimeter. In this way thermal equilibrium in all parts of the mechanism is well established before the starting of the experimental period. The temperature of the calorimeter is measured at frequent intervals and the heating current adjusted to keep the box temperature about equal to its initial temperature, i.e., before starting the flow of water. Other routine such as rechecking the radiometer calibration and checking the weight of the oxygen tank is completed by the time the subject arrives at about 9 A.M.

The subject who has had no breakfast sits in ordinary indoor clothing for 1 hour in the prevailing atmosphere. At 10 A.M. the subject undresses and at the same time measures the temperature of the skin under his clothing. He is weighed immediately after voiding, the rectal thermometer inserted, and is sealed in the calorimeter at about 10.15. The air circulation is started and the preliminary period is begun. It requires about 30 minutes to allow all the parts of the calorimeter to come again into thermal equilibrium. At 10.45 the subject makes his first measurement of surface temperature and box radiation temperature. The radiometer is pointed

at all parts of the inner surface of the calorimeter, and the readings are weighted according to the surface area of the calorimeter part. The side walls are usually at the same radiation temperature, but the top of the calorimeter is about 0.5°C . cooler than the rest of the box. It has been found that the weighted average of all readings is within 0.1°C . of the average air temperature which is measured by six resistance thermometers located in various parts of the box.

At about 11 A.M. the first experimental period is begun, and during this basal period the subject remains as quiet and motionless as possible. Immediately after the start of the second period the skin and box wall temperatures are again measured. The second and third periods are quite often used for studying the effects of exercise, forced air currents, or chilling, so that the surface temperature may be measured several times during these periods. At the end of the experiment the skin and wall temperatures are again measured. From the data thus obtained all the factors of heat production, heat elimination, and the partition of the latter are obtained, as well as a record of the effect of certain external factors on the skin temperature.

The following is a sample of the calculation of the heat produced and heat eliminated, and the factors of radiation, convection and vaporization.

Date: March 13, 1935

Subject: E.F.D.B., basal experiment, nude

Time: 11.15 A.M. to 12.15 P.M.

Age 52, weight 74.74, height 178 cm.

Total surface area = 1.96 sq.m.

Effective radiating surface area = 1.54 sq.m.

Average calorimeter temperature = 27.40°C .

Average calorimeter humidity = 25%

Heat produced

	<i>Absorbed grams</i>	<i>Correction for residual air</i>	<i>Consumed or produced grams</i>	<i>R.Q.</i>	<i>Heat produced</i>
CO_2	25.02	+ 1.11	23.16		
O_2	21.81	— 1.14	20.67	0.815	68.65
H_2O	35.95	— 0.53	35.42		

Heat eliminated

Weight of water flowing through calorimeter = 21.41 kg.

Average temperature of ingoing water = 20.81°C.

Average temperature of outgoing water = 23.48°C.

Change in box temperature (thermal capacity = 16 kg. per degree) = 0.03° drop

Total heat absorbed = 57.16 — 0.48 = 56.68 Calories

Heat lost by vaporization = 20.68 Calories

Total heat eliminated = 77.36 Calories

Body heat storage

	<i>Start</i>	<i>End</i>
Rectal	37.25	37.10
Average skin	33.02	32.81

Heat storage observed = 77.36 — 68.65 = — 8.71 Cals.

Heat storage calculated from rectal alone = — 9.54 Cals.

Heat storage calculated from 0.8 R + 0.2 S = — 10.32 Cals.

*Partition of heat eliminated**Skin temperature*

	<i>Start</i>		<i>Finish</i>		<i>Radiation average Cals. per sq.m. per hour</i>
	<i>Temp.</i>	<i>Temp. weighted</i>	<i>Temp.</i>	<i>Temp. weighted</i>	
Head	35.0	245	35.0	245	
Arms	33.3	466	32.7	458	
Hands	34.1	171	33.8	169	
Feet	30.6	214	30.4	213	
Legs	31.8	413	31.5	409	
Thighs	31.9	606	31.6	600	
Trunk	33.9	1187	33.9	1187	
Average skin temperature		33.02		32.91	29.7

Total heat lost by radiation = $29.7 \times 1.54 = 45.70$ Cals. = 58%

Total heat lost by convection = $56.68 - 45.7 = 11.00$ Cals. = 15%

Total heat lost by vaporization = 20.66 Cals. = 27%

Total 77.36 Cals. = 100%

SUMMARY

The total radiation from the surface of the human body can be determined by means of the Hardy radiometer making due allowance for the effective radiating surface which is smaller than the total surface. The respiration calorimeter of the Russell Sage Institute of Pathology in one set of measurements determines the heat lost in vaporization. By an independent method it gives accurate figures for the heat lost in

radiation plus convection. This makes it possible to determine by subtraction the heat loss by convection and to partition the three important channels of heat loss. Inasmuch as calculations are based on heat loss and not heat production the heat storage in the body is not employed in determining partition of heat loss. It can be calculated from the difference in heat production and heat loss.

It is believed that the combination of respiration calorimeter and radiometer has provided the first accurate measurement of normal convection loss.

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BASAL METABOLISM, RADIATION, CONVECTION AND VAPORIZATION AT TEMPERATURES OF 22 to 35°C.¹

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SIX FIGURES

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There are few studies in the literature dealing with attempts to separate radiation from convection. The first important work was done by Aldrich ('28), and later Bohnenkamp and Ernst ('31) and his associates stimulated research in this field. Burton and Bazett ('36) and Burton ('34) have made valuable contributions. Recently Winslow, Herrington and Gagge ('36, '37 a, b) using the Hardy radiometer have published some important results. Our colleagues, the heating and ventilating engineers, have for several years realized the necessity for this differentiation and a new method of heating rooms based on low temperature radiation has been introduced into England by Barker ('32). The studies of Houghten, Teague, Miller and Yant ('35); and Yaglou ('26), have been of great practical service though their results cannot be compared with ours since they did not measure heat loss and their subjects were clothed and moderately active. There have been many reports on the effect of exposing animals and clothed and naked persons to various temperatures. The literature is summarized by Lusk ('28), Deighton ('33), Du Bois ('36), and by Swift ('32), who gives an excellent discussion of shivering.

¹ Clinical Calorimetry no. 50.

The physical factors in body temperature maintenance and heat elimination are well reviewed by Deighton.

Our experimental technic has been described in the previous paper (Hardy and Du Bois, '38). Only two normal subjects have been fully studied as it seemed wiser to make many experiments on two people rather than few observations on many individuals. It has required almost a year's work of four experimenters to complete the observation on one subject.

EXPERIMENTAL SUBJECTS

E.F.D.B., physician, born June, 1882, age 54, height 179 cm., weight 77.5 kg., average build, has been the subject of many respiration experiments since 1909 (Du Bois, '36). In most of these he lay lightly clothed in a calorimeter at comfortable air temperature. His metabolism has been unusually constant, falling slightly with advancing age. His weight has not varied significantly and his mode of life has been uniform. He exercises regularly, running about $1\frac{1}{4}$ miles every morning before breakfast except, of course, on experimental days. During the last 5 or 6 years there has been a slight irregularity of the heart due to nodal rhythm, a condition not infrequent in persons with slow heart rates. This irregularity disappears in exercise and in no way affects the circulation. X-rays of the extremities show the slight calcification of the vessels consistent with his age. He has lived almost all his life in New York and Massachusetts and stands cold air and cold water as well as the average man. His response to heat by sweating is about average. In the experiments made by C.G. and F.G. Benedict and Du Bois ('25) he was exposed to very dry, very hot air entering an oil cloth bag at a temperature of 94°C . His metabolism was increased only 7% in spite of the fact that his feet and legs were almost unbearably hot.

J. D. H., physicist, born in Texas, 1904, age 33, height 168 cm., weight 67 kg., is of a shorter, stockier build than D, with a little more subcutaneous fat. He has always been athletic and he plays a hard game of squash about three times a week.

His health is good except for occasional colds. Physical examination shows normal heart and lungs. Until the age of 23 he lived in Mississippi, since then has been in Maryland, Michigan and New York. He is about as well adjusted to cold as the average New Yorker. His sweating response to warm environments or to exercise is about average.

EXPERIMENTAL CONDITIONS

The experiments were started in the neutral zone of temperatures, 27°C. to 29°C., and then extended to the extremes of heat and cold with repetitions and interruptions so that the final curve would not be affected by any possible seasonal variation if such exists. Experiments were made during the spring and winter months of 1935, 1936 and 1937. The subjects came to the laboratory having had no breakfast except perhaps a cup of caffeine free coffee or a cup of hot tomato juice. In the winter they were normally dressed for the trip to the hospital. Arriving at about 9 A.M., they sat dressed in ordinary clothing until about 10 A.M. when they undressed. As their clothes were removed measurements were made of skin temperature. About 10 A.M. they were placed in the calorimeter and about 11 o'clock the experiment began. Tests on these two men and a number of others have shown that at the lower range of temperatures the skin cooled rapidly for the first 90 minutes and then fell much more slowly. In the warmer air range the fall of skin temperature was much less marked and uniformity was rapidly attained. In the cooler experiment the subjects were cold and slightly uncomfortable during the preliminary period and first basal hour. Eventually the combination of cold air and absolute quiet brought about distinct chilly sensations with an involuntary tensing of muscles which involved no visible shaking. This gradually increased over a period of 5 to 20 minutes before there was a violent chill with chattering of the teeth and clonic movements of the extremities which shook the whole box. It is interesting that both men maintained their metabolism at the ordinary basal levels until a few minutes before the chill began and

they were able to warn the observers about 10 to 15 minutes before the approaching chill so that a second experimental period could be started which would include the onset of shivering. At the highest temperatures the subjects were sweating from the beginning of the preliminary period to the end of the experiment. At a certain critical warm temperature there would be a constant sweating in the axillae with transient outbreaks of sweat over the body when the subject expended the extra two or three calories involved in making the surface temperature readings. Except for these measurements and for occasional turnings from side to back the men lay almost motionless during the basal hours. Sometimes they dozed lightly for a few minutes.

After a satisfactory basal hour had been obtained the second and third periods were used for a study of the effects of exercise or of an electric fan. The data of all the basal experiments on the nude subjects are given in table 1; other basal periods, associated with exercise and chill experiments, will be found in subsequent papers. The experiments are arranged in order of increasing calorimeter temperature, and the first part of the table is concerned with gaseous exchanges involved in calculation of heat production. The later columns contain the thermal data and the heat eliminated. In this report all the nude basal periods are considered together.

DISCUSSION

Skin, rectal and average body temperatures

Skin temperature. The rectal and surface temperatures of the various parts of the body at different calorimeter temperatures are shown in figures 1 and 2. Each vertical set of readings was made on a different day but the results are surprisingly consistent. It happened in both subjects that the line for the temperature of the hands ran very close to the weighted average of the whole surface. If this should be the case for a large number of persons it would be a point of considerable practical value. In warm atmospheres the extremities and other parts of the skin surface have temperatures ranging within $\pm 1^\circ$ of the surface of the trunk.

TABLE 1

Two-hour basal experiments on subjects H and D

SUBJECT, DATE, WEIGHT, REMARKS	END OF PERIOD	O ₂	S	R _Q	H ₂ O	URINARY PER HOUR	PULSE RATE	TOTAL HEAT PRODUCED	CALORIMETER TEMPERATURE	CALORIMETER HUMIDITY	RECTAL TEMPERATURE	TOTAL HEAT ELIMINATED	SKIN TEMPERATURE		RADIATION	CONVECTION	VAPORIZATION
													Time	Tempera- ture			
JDH. Feb. 18, 1937, 66.7 kg. 1st period basal; 2nd period quiet; cold at end	11:08							<i>Cal.</i>		%	36.78	<i>Cal.</i>	10:48	32.9		%	
	12:08	18.9	16.9	0.81	24.5	0.51	53	56.0	25.40	23	36.03	74.2	12:20	31.9		11	19
	1:08	23.4	20.4	0.83	26.1		49	68.2	25.38	22	36.66	77.0	1:20	31.6		19	20
JDH. Feb. 4, 1937, 67.2 kg. Pajamas and 2 pair socks Both periods basal. No surface measurements	10:58										37.08						
	11:58	21.2	17.8	0.87	25.9		59	59.9	26.53	25	37.01	61.9					
	12:58	21.3	18.3	0.85	25.2	0.42	54	61.5	26.54	25	37.04	61.4					
JDH. Feb. 16, 1937, 66.9 kg. Both periods basal	11:23										36.72		11:00	33.5			
	12:23	24.0	20.1	0.87	27.2		52	67.5	26.91	22	36.63	68.1	12:40	32.9		10	23
	1:23	16.1	15.9	0.74	26.4	0.70	51	51.3	26.80	20	36.63	66.2	1:35	32.8		10	23
JDH. Feb. 11, 1937, 66.8 kg. 1st period basal. 2nd period basal	11:09										36.76		10:49	33.6			
	12:09	19.4	17.4	0.81	27.3		54	57.5	27.67	23	36.71	69.3	12:25	33.0		15	23
	1:09	22.3	18.9	0.86	28.6	0.51	54	63.4	27.61	22	36.76	68.0	1:20	32.8		17	25
EFDB. Feb. 6, 1936, 77.6 kg. 1st period basal. 2nd period basal	10:42										37.14		10:20	32.7			
	11:42	23.8	20.2	0.86	37.9		57	67.9	27.71	28	36.95	83.4	11:45	32.8		20	27
	12:42	24.3	21.3	0.83	40.3	0.56	52	70.9	27.64	27	36.88	83.6	12:44	32.6		20	28
JDH. Feb. 9, 1937, 66.7 kg. 1st period basal. 2nd period quiet	10:55												10:30	34.1			
	11:55	19.4	17.8	0.79	32.6		60	58.6	28.58	25		65.8	12:10	33.2		13	29
	12:55	23.6	20.5	0.84	33.8	0.55	60	68.4	28.60	23		66.7	1:05	33.7		15	30

In all experiments of this group the head had the highest skin temperature. The extremities, especially the feet, began to show a precipitate fall in temperature when the calorimeter air was lower than 28°C . Also at about this point the subjects

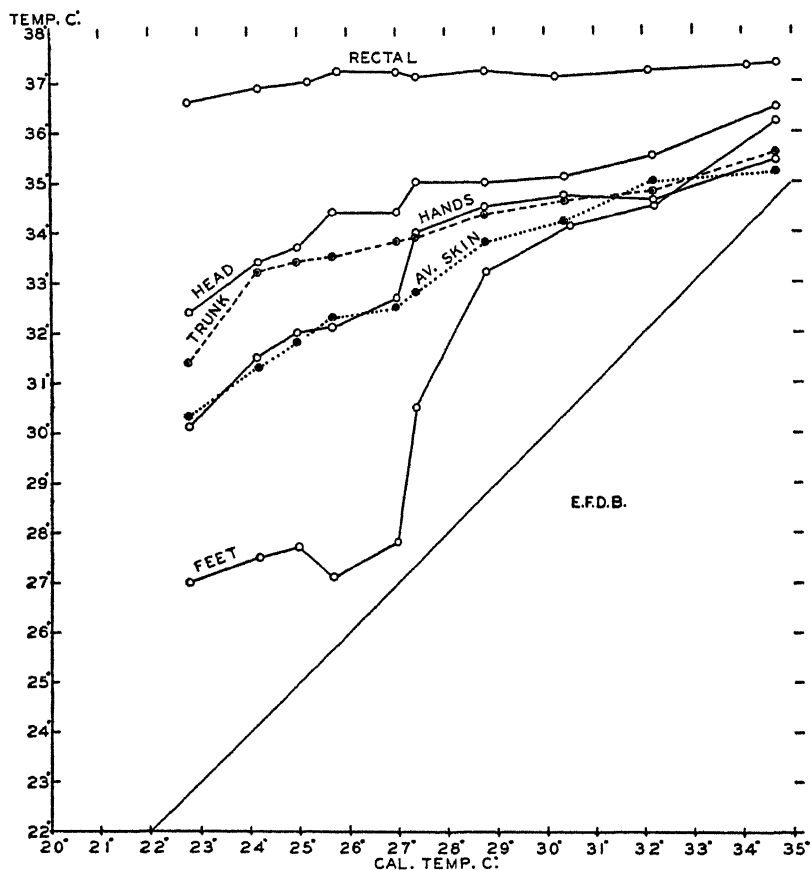


Fig. 1 Skin and rectal temperatures of subject D, during basal experiments at different environmental temperatures.

began to feel cool. In cold environments the temperature of the feet was low, often the surface of the toes was colder than the air on account of the large amount of surface for vaporization, the low heat production of the bones, joints and tendons, poor blood supply and the long distance from the

'central heating apparatus.' The other end of the body, the head, was relatively little affected by the cold air. It has a better blood supply and is accustomed to nakedness in cold weather. The trunk and hands occupy intermediate positions. Although in warm environments the skin temperature rapidly establishes an equilibrium level, upon exposure to cold (Cal.

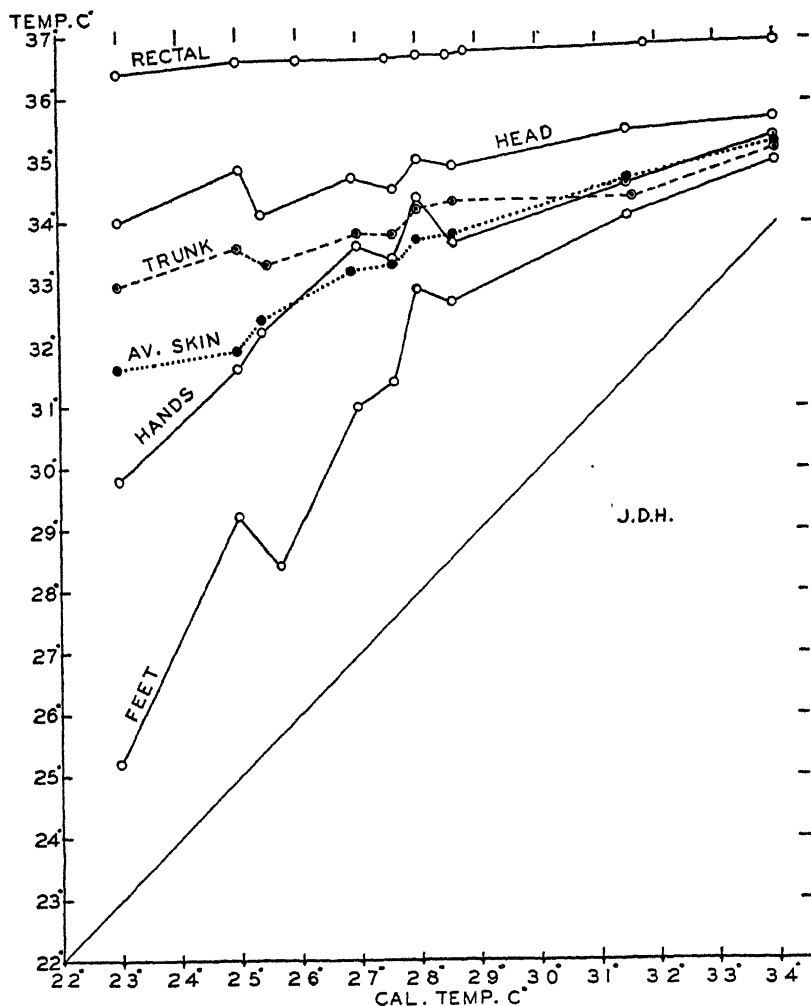


Fig. 2 Skin and rectal temperatures of subject H, at different environmental temperatures.

Temp. less than $26^{\circ}\text{C}.$) the skin temperature falls until the subject shivers. It is difficult to speak of a 'level' of skin temperature under these conditions, and the values of skin temperature given for these experiments are the average readings for the beginning and end of the first basal hour. The tendency of all parts of the skin surface to assume the same temperature in warm environments is demonstrated in both subjects.

For every degree rise in environmental temperature the average skin temperature increases about $0.5^{\circ}\text{C}.$, in the range of temperature from 22° to $29^{\circ}\text{C}.$ At $29^{\circ}\text{C}.$ sweating has increased to the point where its cooling effect on the skin is apparent and higher environmental temperatures cause only relatively slight increases in skin temperature. The maximum value for average skin temperature observed in the basal experiments was $35.3^{\circ}\text{C}.$ It is probable that environments hotter than $36^{\circ}\text{C}.$ would cause a fall in skin temperature. Such an observation has been reported by Wiley and Newburgh ('31). Figure 6 shows the average skin temperatures for subjects D and H and they are almost identical.

Rectal temperature. The rectal temperature of D averaged about $0.4^{\circ}\text{C}.$ higher than that of H. In nearly all experiments the rectal temperature fell during the basal, first period, and the fall was greater in cold environments. The average rectal temperature rose with increased environmental temperature and at $23^{\circ}\text{C}.$ the resting rectal temperature was from $0.6^{\circ}\text{C}.$ to $0.8^{\circ}\text{C}.$ lower than at $34^{\circ}\text{C}.$ Thus, a lower level of rectal temperature of this extent, of itself, will not cause shivering or an increase in heat production.

It is of interest to note that the skin temperature of these two men was about halfway between internal (rectal) and air temperatures. The distances between the lines show the size of the temperature gradients but not their shapes. Some observers, using electrical resistance wires in needles, have found that in parts of the body such as the wrists the subcutaneous temperature may be lower than skin temperature, owing to the cooling effect of the blood in the veins.

Average body temperature. This quantity is of considerable importance from both a laboratory and clinical standpoint. Its importance in calorimetric work is obvious, and from the clinical side the estimation of the average body temperature of patients being treated with hyperpyrexia is very desirable. So far, our observations concern only the resting basal state, and can under these circumstances throw light only on the questions: how well does the rectal or skin temperature represent the average body temperature, and how well does any combination of rectal and average skin temperature represent average body temperature? Barr and Du Bois ('18) made estimates of the changes in average body temperature by subtracting the heat produced in the body from the heat eliminated. Burton ('35) has correlated this difference with the changes in skin and rectal temperature and he arrived at the formula:

$$\text{Average body temperature} = 0.65 \text{ rectal} + 0.35 \text{ skin}$$

He, at the same time, made the observation that the skin temperature was the more important factor even though it is weighted only 35%. Burton's skin thermometer was of a type which would indicate changes rather than the actual skin temperature. The data of the present experiments were studied to determine the relationship between the thermal changes in the rectal cavity, the skin, and the average temperature of the body. It was at once clear that the change in rectal temperature alone would give a fair approximation of the change in average body temperature, although a slightly better approximation could be made using the following formula:

$$\text{Average body temperature} = 0.8 \text{ rectal} + 0.2 \text{ skin}$$

Even after decreasing the weight of the skin temperature term, this factor is still an important one. Du Bois has figured that for a man weighing 70 kg., about 15 kg. is within 1 cm. of the surface. Our equation would lead one to conclude that 14 kg. of the man's mass is to be weighted with the skin temperature.

The simplest way to study the average temperature of the body is to make direct observation of the actual temperature changes by measuring the heat production and elimination. Then

$$-\Delta T_b = \frac{H_e - H_p}{M \times S}$$

ΔT_b = change in average body temperature

H_e = heat eliminated

H_p = heat produced

M = mass of subject

S = average specific heat of body tissues

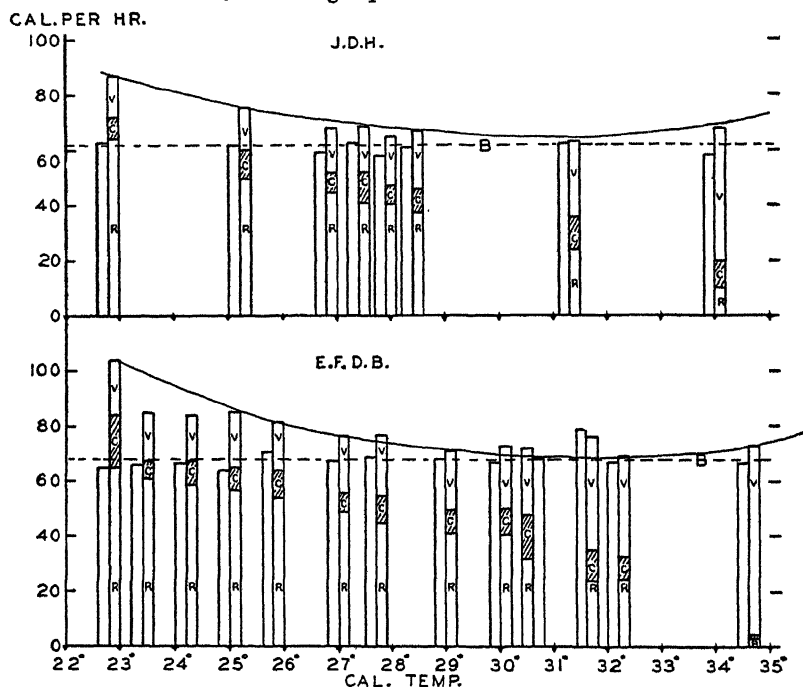


Fig. 3 Changes in heat production and heat elimination for both subjects during the basal periods with calorimeter temperature. Blank columns on left, heat produced; columns on right, heat eliminated; V, heat loss by vaporization; C, heat loss by convection; R, heat loss by radiation.

A value of 0.83 is usually assumed for the average specific heat of the body tissues, and all the other factors except T_b are measured so that this quantity is determined. Correlation of this factor with the temperature measurements on the body can then be attempted. The only bodily temperature

measurements which were made in these experiments were the rectal and average skin temperature. In figure 4 are plotted four sets of combinations of skin and rectal temperature against T_b . The formulae so tested are:

1. $-\Delta T_b = -k \Delta R$
2. $= -k (0.8 \Delta R + 0.2 \Delta S)$
3. $= -k (0.65 \Delta R + 0.35 \Delta S)$
4. $= -k \Delta S$

$$k = 0.83 \times M$$

R = change in rectal temperature

S = change in skin

The change in body temperature was negative in all these experiments; that is, the body temperature was falling. In order to plot the data in the usual way all the signs were reversed.

The relation of rectal temperature change to average body temperature change is seen to be fairly close in these experiments, although the average line does not pass through the origin but about 0.09°C . above the origin. The spread of the values is $\pm 0.15^\circ\text{C}$. By accounting also for the skin temperature with a weight of $0.8\Delta R$ and $0.2\Delta S$, the average curve is above the origin by 0.06°C . and the spread $\pm 0.09^\circ\text{C}$. Using the formula $0.65\Delta R$ and $0.35\Delta S$, the average curve passes 0.05°C . above the origin and the spread is $\pm 0.17^\circ\text{C}$. The skin temperature relation shows a much smaller slope and passes 0.09°C . above the origin with a $\pm 0.17^\circ\text{C}$. spread.

The slope of the average ΔR curve is greater than unity indicating that the body temperature changes more than is shown by the rectal alone. The slope of Burton's formula is less than unity, evidence of too great a weight on the skin temperature. The slope of the $0.8\Delta R + 0.2\Delta S$ is unity and as the spreading of the data is minimal, this formula is taken to best fit these observations. The slope of the skin temperature curve is very much smaller than unity (only 0.25) showing that the body average temperature does not change anything like as much as the skin temperature. The correlation however between the changes in average skin and average body temperature is good enough to show the importance of this factor.

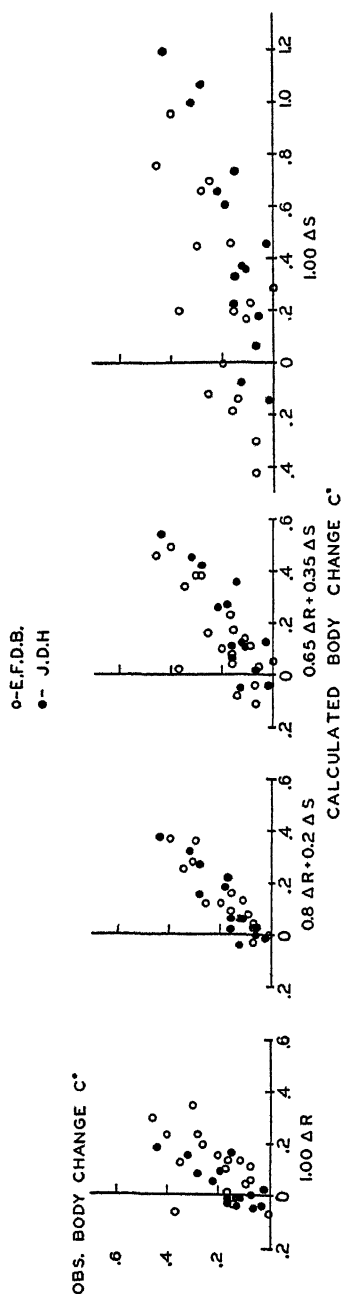


Fig. 4 Relation of observed change in average body temperature to calculated temperature changes from ΔR , changes in rectal temperature, and ΔS , changes in skin temperature.

The fact that none of the curves pass through the origin may possibly be due to a systematic difference between the heat eliminated and heat produced of 2.5 calories, but is more probably due to a slight fall of temperature ($0.06^{\circ}\text{C}.$) of the rectal cavity associated with muscular relaxation during the first basal period. As will be apparent later, the strict application of any single formula for average body temperature may lead to error, especially during exercise when the skin temperature may fall several degrees in spite of increased body temperature and vaso-dilatation. Patients in chills and fever sometimes show higher skin temperature than rectal temperature, in which case the average body temperature should be related more to the skin temperature. So far as we have studied the matter, no one formula can be used under all conditions.

Average specific heat of body tissue

Assuming that the formula proposed for average change in body temperature represents this value fairly closely, an estimation of the specific heat of the body may be made. Evidently,

$$\Delta C = M \times S \times \Delta T_b = H_p - H_e,$$

where ΔC = heat lost from the body
 M = mass of the body
 S = average specific heat of body
 ΔT_b = average change in body temperature
 H_p = heat produced
 H_e = heat eliminated

Then, by putting in the formula for average body temperature change,

$$S = \frac{H_p - H_e}{M(0.8\Delta R + 0.2\Delta S)}$$

Graphical estimation of S may be had by plotting $H_p - H_e$ against $M(0.8\Delta R + 0.2\Delta S)$, as is done in figure 5.

The slope of the best straight line through these points is the desired value of S , and the extreme values come out 0.83 and 0.72 with the most probable value 0.78. Burton ('35) made similar estimates on his subjects and arrived at the

conclusion that S lay, within wide limits, between 0.7 and 0.9. He also correctly pointed out that S would vary considerably in fat persons and in thin persons.

OBS. BODY CHANGE CAL.

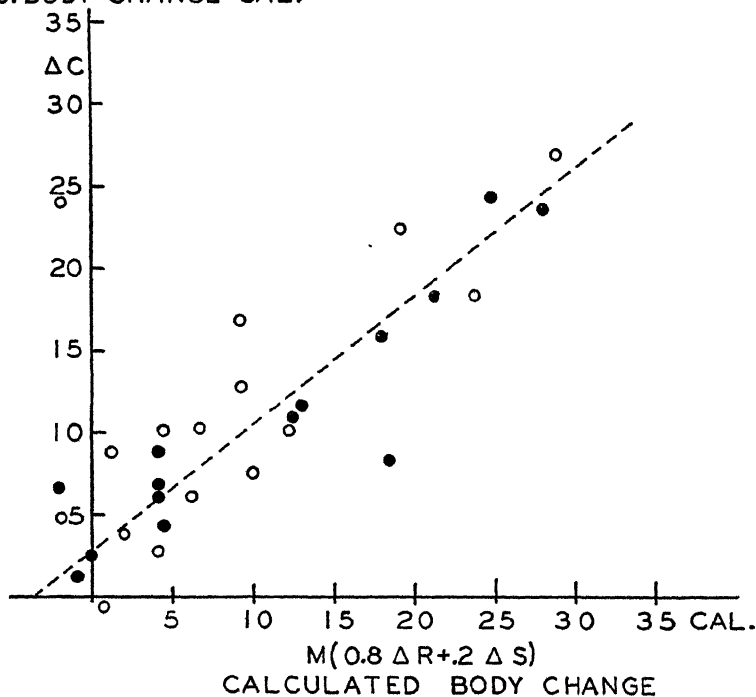


Fig. 5 Graphical estimation of specific heat of body from measured body heat change and measured body thermal change.

Heat production

Figure 3 summarizes the results on the subjects D and H. They bring out clearly the difference between heat production and heat elimination. The blank columns to the left show the heat production as determined from the oxygen consumption, nitrogen elimination, and R.Q., using the method of indirect calorimetry. During the periods of observation which have been called basal, the heat production of E.F.D.B. was 69 cal. per hour ± 3 cal., or 34.9 cal. per

square meter per hour. This, according to the Aub and Du Bois standards, is -7% . For J.D.H. the heat production was 62 cal. per hour ± 3 cal., or 35.0 cal. per square meter per hour. This is, according to the same standards, -12% . In the range of temperatures down to 22°C . it was possible for both subjects to remain in a basal state for about 2 hours, that is, during a preliminary and first experimental period. Actually, the heat production tended to decrease slightly in cold atmospheres, possibly in accord with van't Hoff's law. Temperatures up to 35°C . seemed to have no effect upon heat production.

In cold experiments the periods immediately preceeding a chill were not considered basal if the subjects were restless. During this time the subjects usually felt under considerable mental tension although the effort required to prevent the onset of chill was not found to increase oxygen consumption. At times, during some of these periods, involuntary muscular contractions occurred. In all cases in which increased heat production occurred, muscular 'tensing' or actual shivering appeared, and no increase in oxygen consumption as evidence of a purely chemical response to the exposure to cold (Rubner's chemical regulation of body temperature) was observed.

Heat elimination

Heat elimination, shown by the right hand columns in figure 3 with shaded areas, is equal to the heat production only in the relatively narrow neutral zone between 28°C . and 32°C . The thermal conditions of the atmosphere in this zone with about 30% relative humidity are such as to provide a natural escape for the body heat, under basal conditions, at exactly the rate of its production. In this 'thermally neutral zone' the subjects are comfortable throughout the experiment, although any amount of activity will usually produce sweating.

As the environmental temperature is lowered (down to 22°C .) the heat elimination becomes larger than the heat

produced by about 3 cal. per hour for each degree drop in temperature. The body temperature therefore falls as is evidenced by the rapid fall in skin temperature and the slow drop in rectal temperature. If 15 kg. of tissue falls 1°C . it will give off 8 to 12 calories, depending on the relative content of water (Sp.Ht. = 1.0) and fat (Sp.Ht. = 0.5) in the skin. The 8 to 12 calories thus eliminated were not produced by the metabolism during the basal periods.

The heat elimination also increases with rising environmental temperatures. This would be expected, since the body will absorb energy from the environment at temperatures greater than 35°C . However, no experiments were made at environmental temperatures over 35°C . as the calorimeter absorber mechanism is not adequate for the removal of such large amounts of moisture. The rise in heat loss between 31°C . and 35°C . is probably due to slight overcooling by the sweat glands.

The actual number of calories lost from the body through vaporization, V, convection, C, and radiation, R, are also shown in figure 3. Radiation, which depends solely upon the skin surface area and temperature and the environmental objects' temperature, increases from 0 at 35°C . up to more than the total heat production at 22°C . Vaporization decreases from about 80 calories per hour at 35°C . to from 15 to 19 calories per hour at 22°C .

Convection, which is most difficult to measure directly, is arrived at by the difference between the total heat absorbed by the calorimeter and the loss due to radiation. The convection loss is small, ranging from 8 to 16 cal., for nude subjects under basal conditions. The convection loss does not seem to depend to any great extent upon the temperature of the environment except at air temperatures near the skin temperature. Convection loss from inanimate objects is known to depend upon the size, shape, surface and position relative to other objects as well as upon difference in temperature between objects and air and air movement. In these experiments the natural air movement was so small that it

could scarcely be felt by a naked man. The position of the man in the box was such as would hamper free air movement, a fact which would help account for small convection losses. By far the most important factor in convection in our experiments is air movement. Convection is markedly increased by the slightest movement on the part of the man and even the movements of the ribs in breathing and the currents of air in and out of the nostrils cause some turbulence not found with inanimate objects. Slight movements of the extremities which scarcely affect the metabolism cause increased convection. Calorimeter humidity and the vaporizing of water from the skin have no demonstrable effects on convection. Rapid movements of the subject may cause convection to rise to almost as high a value as that due to the blast of air from an electric fan.

The percentages of heat lost through vaporization, convection, and radiation are shown in figure 6. It will be seen that the curves for vaporization and radiation are almost complementary except for the fact that vaporization never becomes zero. Radiation has its highest values at about 22°C. to 23°C. where it amounts to about 70% of the total heat loss. With increasing temperature radiation plays a smaller role and becomes zero at 35°C. Radiation is equal to vaporization in the upper 'neutral zone,' at 31°C.

Convection remains about constant, decreasing in importance in cold environments and in warm environments. The reason for the decrease in cold temperatures may be due to the fact that the subjects were concentrating on remaining quiet in effort to ward off the onset of chill. The rise in convection in the 'neutral zone' is partly due to increased ventilation in the calorimeter to take care of the moisture. The decrease in the proportion of heat loss due to convection in warmer environments is due to the smaller gradients between skin and air. Convection becomes zero at 35°C.

It should be noticed that radiation has its maximum proportion of heat loss and convection its minimum when the subject is lying motionless, i.e., in the basal state. Almost

any other condition tends to decrease the porportion due to radiation and increase that due to convection.

The limited ability of the vaso-constrictor mechanism of the body to provide sufficient protection against cold is demonstrated by the fact that the skin temperature falls only one degree when the environment has fallen two degrees. The

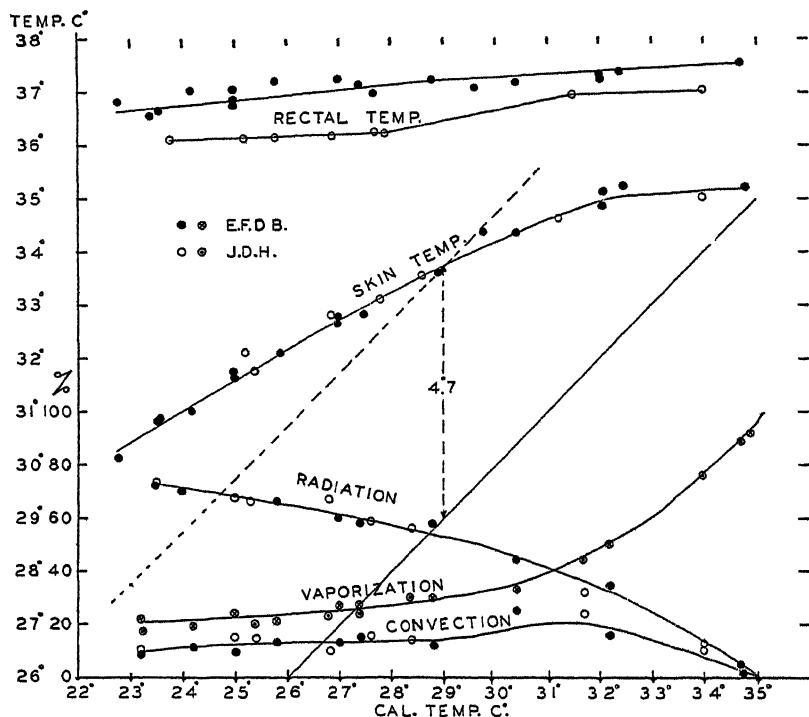


Fig. 6 Effect of calorimeter temperature upon rectal temperature, average skin temperature, and percentages of heat loss from radiation, vaporization and convection.

thermal gradient from the skin to the environment, at the lower temperature limit of the neutral zone, is 4.7°C. This is the maximum gradient over which the body can maintain its temperature without increase in heat production. Therefore, as the environmental temperature is lowered, the skin should drop so as to maintain a gradient no greater than this,

and should follow the dashed line in figure 4. The height of the true skin temperature above the dashed line is proportional to the rate at which the body temperature is falling.

Pulse, nitrogen elimination and total R.Q.

An increase in heart rate was observed in both subjects as the temperature of the experimental environment was raised. Subject D, who has a slow pulse, evidenced a linear rise from about 52 to 59 beats per minute. Subject H showed a rise from about 52 to 66. This is perhaps associated with the dilation of the skin vessels at the higher temperatures.

An observed fall in nitrogen elimination with increasing calorimeter temperature from about 0.7 to 0.3 gm. per hour is perhaps due in part to increased sweating, and yet it is apparent at temperatures where vaporization was changing but little. No attempt was made to control the protein intake the day before the experiment. Under similar dietary conditions in previous years control D, when studied in the calorimeter, eliminated rather uniformly about 0.5 gm. nitrogen per hour. Although Swift ('32) reported a similar effect, the evidence is not sufficient to indicate any specific change in nitrogen metabolism at varying temperatures. No temperature effect on the total R.Q. could be found.

SUMMARY AND CONCLUSIONS

It has been possible for the first time to measure quantitatively the total heat loss and the proportions due to radiation and convection from men exposed to various atmospheric conditions.

Two normal men were studied naked under basal conditions in the respiration calorimeter of the Russell Sage Institute of Pathology at temperatures between 22°C. and 35°C. The heat lost in vaporization was measured by weighing the water. The heat of radiation was determined by means of a Hardy radiometer. Convection was found by difference. Calculations were based on heat elimination and not heat production. Heat storage, which was not a factor in the

basic calculations, was estimated from rectal and surface temperature changes and was measured by the difference between direct and indirect calorimetry. Between 22°C. and 35°C. the average temperature of the skin lay about midway between that of the air and that of the internal parts of the body. At the lower temperatures the surface layer of the body cooled rapidly and heat elimination greatly exceeded heat production. Radiation accounted for about 70% of the total loss at 22°C. to 26°C., but this percentage fell rapidly to zero as skin and air temperatures approached each other. Vaporization dissipated 18% to 30% of the heat at the lower air temperatures but accounted for about 100% at 35°C. Convection remained fairly uniform at about 15% until the air temperature rose above 32°C. Convection is significantly increased by slight movements of the body or the air.

The basal metabolism of both naked subjects was level throughout the range of air temperatures from 22°C. to 35°C. At the lower temperatures the temperature of the skin and subcutaneous tissues dropped rapidly. After about 2 hours the men felt distinctly chilly and there was a tensing of the muscles which raised the metabolism slightly. After 10 or 15 minutes this was followed by a shaking chill. The fact that metabolism was not increased until a few minutes before the chill throws doubt on the existence of Rubner's 'chemical regulation.' At the higher temperatures increased sweating took care of the heat loss when radiation and convection were abolished.

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THE EFFECT OF UREA ON THE HUMAN RESPIRATORY EXCHANGE AND ALVEOLAR CARBON DIOXIDE¹

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Urea constitutes the major nitrogenous excretion in the urine in the metabolism of protein and, therefore, has been the subject of many studies as to whether the transference of this substance from the blood into the urine is the cause of the specific dynamic action of protein. Lublin ('28) and Borsook and Winegarden ('31 a, b, c) came to the conclusion that there was an increased energy output due to the ingestion of urea, whereas Tangl ('11), Lusk ('12), Grafe ('15), Kocher and Torbert ('32), di Frisco ('33), Eaton, Cordill and Gouaux ('35), and Rajzman ('36) found negative results so far as any effect of ingestion of urea on the total metabolism is concerned. Ingestion of urea by mouth should cause no change in the respiratory quotient, as no oxidation is involved in its passage in an unchanged form through the body. Adolph ('25), however, found that there was an increase in the alveolar carbon-dioxide tension when urea was given to human subjects. In a general study of the various factors affecting the respiratory quotient, this investigation has been made to determine whether with a change in the alveolar carbon dioxide there would be a change in the respiratory quotient and at the same time to add another study on the question of the effect of urea on the total metabolism.

¹A preliminary report of this material was presented at the meeting of the American Chemical Society, Rochester, N. Y., on September 7, 1937.

Methods. The respiratory quotient was measured by the open circuit apparatus of Carpenter and Fox ('31) in one group of experiments and by the Benedict helmet ('30, '33) and open circuit apparatus in another group. When the apparatus of Carpenter and Fox was used, alveolar air samples were collected every 7 to 8 minutes in parallel with the respiratory exchange measurements by the arrangement of Carpenter and Lee ('33).

Procedure. The subject was H. C. W., 22 years of age, 68.0 kg. in weight, and 175 cm. in height. The experiments were all made with him in a sitting position and in the post-absorptive condition. After the preliminary rest, measurements were made in three periods of 15 minutes' duration each, and the dose for the day was then given to the subject. When the apparatus with mouthpiece was used, the ingestion of the dose was followed by three 15-minute periods of measurement out of each succeeding hour (the first three quarter-hours) until a total of $3\frac{1}{2}$ hours had elapsed after the dose was given. In one series of urea experiments the sequence of periods was changed in that only two 15-minute periods of measurement immediately followed the dose and then groups of three 15-minute periods followed which were separated by 15-minute intervals without measurement. In this way the periods omitted in the grouping first described were covered in this particular series. Two series were also made, one with water and one with urea and water, in which only one 15-minute period of measurement was made in each half hour and the subject drank water and urinated every half hour. When the helmet was used, there were three base-line periods as in the mouthpiece experiments, but after the dose the measurements of the respiratory exchange were made continuously in 15-minute periods without interruption for 3 hours. By this procedure the effect of interruption of observations was avoided and the true continuous changes in the gaseous exchange and the respiratory quotient were determined. Four types of experiments were made. The first was with no dose, in which the whole routine was carried out

as in the other groups; the second was with 400 or 500 cc. of water to serve as a control for the urea experiments; and the third and fourth series were with 30 and 40 gm. of urea in 400 and 500 cc. of water, respectively.

ALVEOLAR CARBON DIOXIDE

The results of the determinations of the alveolar carbon dioxide are given in table 1. The table includes the number

TABLE 1

Human alveolar carbon dioxide in experiments with no dose, water and urea

	NO DOSE	400 CC. WATER	30 GM. UREA ¹	40 GM. UREA ²	40 GM. UREA ²
	%	%	%	%	%
Number of experiments	4	4	5	3	3
Base line	5.73	5.94	5.92	5.69	5.72
<i>Minutes after ingestion</i>					
0- 15	5.72	5.78	5.96	5.91	5.88
15- 30	5.70	5.75	6.12	6.07	6.05
30- 45	5.65	5.83	6.21	6.07	..
45- 60	6.01
60- 75	5.75	5.93	6.09	5.97	5.97
75- 90	5.77	5.88	6.06	6.06	5.98
90-105	5.73	5.93	6.07	6.03	..
105-120	5.92
120-135	5.75	5.75	5.91	5.83	5.82
135-150	5.68	5.77	5.89	5.86	5.81
150-165	5.66	5.81	5.83	5.75	..
165-180	5.82
180-195	5.68	5.78	5.86	5.81	5.86
195-210	5.71	5.77	5.87	5.75	5.78

¹ In 400 cc. of water.

² In 500 cc. of water.

of experiments in each series, the average base-line values for the three 15-minute periods preceding the dose for the day, and the averages by 15-minute periods for 3½ hours following the dose. In the first four series, the periods of 45 to 60, 105 to 120, and 165 to 180 minutes had no observations of the alveolar air as the subject rested in these periods. In the last column the series covering these missing periods is given.

With no dose there was a uniform alveolar carbon dioxide throughout the series, as the range was only from 5.65 to 5.77%, including the base-line value. The original protocols show that even in the average values at every 7 to 8 minutes the range was only 5.61 to 5.82%.

In the series with 400 cc. of water there was a significant fall in the alveolar carbon dioxide in the first three quarter-hours after the water was taken. In the second hour there was a return to practically the base-line value. In the third hour and the first half of the fourth hour there was again a fall. These changes are significant, as the range of the seven average values for every 7- to 8-minute interval in the base-line three quarter-hours was from 5.85 to 6.02%, whereas in the seven average values of the first hour after the dose the range was from 5.71 to 5.88%. Also, in the last 1½ hours, the range of twelve average values at 7- to 8-minute intervals was from 5.71 to 5.89%. At the present time no satisfactory explanation can be given as to the cause of this change in the alveolar carbon dioxide after the ingestion of 400 cc. of water.

With 30 gm. of urea there was a definite rise in the alveolar carbon dioxide that began in the second quarter-hour after ingestion and lasted through the second hour. The highest average value was 6.21% in the third quarter-hour as compared with 5.92%, the average of the three quarter-hours of the base-line group. At the twenty-third, thirty-second, thirty-eighth and forty-seventh minutes of the measurements after ingestion the average values were 6.16, 6.20, 6.14 and 6.28%, so that the rise shown by the average values 6.12 and 6.21 in the second and third quarter-hours is significant. With 40 gm. of urea there was an even more marked rise, for, although numerically the average values after ingestion were close to those after 30 gm., the base-line value with 40 gm. was 5.69 compared with 5.92% with 30 gm. The rise continued for 2½ hours. When the spacing of the periods was changed so that the omitted quarter-hours in the first series with 40 gm. were covered, the percentage of alveolar carbon dioxide rose to the same level and the rise lasted about the

same length of time. It is evident from these two series with 40 gm. of urea that the 15-minute intermission produced no effect on the alveolar carbon dioxide in the periods immediately following the intermission. The rise in alveolar carbon dioxide after the ingestion of urea by man found by Adolph ('25) is, therefore, fully confirmed.

TABLE 2

Average changes from the base line in the human R.Q. and oxygen absorption in no-dose, water and urea experiments. (Open circuit apparatus with mouthpiece)

Number of experiments	NO DOSE		400 CC. WATER		30 GM. UREA ¹		40 GM. UREA ²	
	R.Q.	O ₂ per minute	R.Q.	O ₂ per minute	R.Q.	O ₂ per minute	R.Q.	O ₂ per minute
	4	cc.	4	cc.	5	cc.	3	cc.
Base line	0.81	263	0.80	263	0.82	254	0.80	254
<i>Minutes after ingestion</i>								
0- 15	0	-3	0	+8	-0.02	+9	-0.02	-4
15- 30	+0.01	-2	+0.01	-3	-0.02	0	-0.01	-6
30- 45	+0.01	-8	+0.01	+1	0	+2	0	-6
60- 75	+0.01	-3	0	-1	+0.01	+6	+0.01	-3
75- 90	+0.01	-1	+0.01	-2	+0.02	-1	+0.04	-3
90-105	+0.01	-1	+0.02	-1	+0.02	-5	+0.04	+1
120-135	+0.01	-5	0	0	0	+3	+0.02	-11
135-150	0	-7	0	-1	+0.01	-2	+0.03	-8
150-165	+0.01	-2	+0.01	-5	+0.02	0	+0.03	-4
180-195	-0.02	-7	-0.01	-6	-0.01	+8	0	-13
195-210	-0.01	-8	+0.01	-8	-0.01	+9	+0.02	+4

¹ In 400 cc. of water.

² In 500 cc. of water.

OXYGEN ABSORPTION

The effect of ingestion of urea on the total metabolism and, therefore, on the oxygen absorption is a subject that has occupied the attention of a number of workers, and the results obtained by them have not agreed with one another. Although the primary purpose of the study here reported was not to determine the effect of urea on the oxygen absorption but rather the relation of the changes in the respiratory quotient to those of the alveolar carbon dioxide, the results are uniformly so definite that it has seemed advisable to include

them. The values obtained with the mouthpiece are given in table 2, and those with the Benedict helmet in table 3. Briefly stated, the results showed no rise in the oxygen absorption after the ingestion of 30 or 40 gm. of urea for a period of 3 to 3½ hours. There was a slight fall after urea in the second and third hours, although the maximum change from the base-line value was only 5%, that is, —13 cc. in the period of 180 to

TABLE 3

Average changes from the base line in the human R.Q. and oxygen absorption in no-dose, water and urea experiments. (Benedict helmet apparatus)

	NO DOSE		400-500 CC. WATER		30 GM. UREA ¹		40 GM. UREA ²	
	R.Q.	O ₂ per minute	R.Q.	O ₂ per minute	R.Q.	O ₂ per minute	R.Q.	O ₂ per minute
Number of experiments	3	cc. 3	3	cc. 3	2	cc. 2	3	cc. 3
Base line	0.81	248	0.83	245	0.83	243	0.80	243
<i>Minutes after ingestion</i>								
0- 15	-0.01	-2	0	-2	-0.01	-1	-0.02	+1
15- 30	+0.01	+2	+0.02	-7	0	+3	+0.01	-3
30- 45	+0.01	-4	+0.02	-8	+0.01	+5	+0.01	-2
45- 60	+0.01	-3	+0.01	+1	+0.04	+5	+0.02	-1
60- 75	+0.01	-4	+0.01	-3	+0.02	-6	+0.03	+1
75- 90	+0.01	+1	+0.01	-4	+0.03	-3	+0.04	-5
90-105	+0.02	-4	0	+1	+0.02	-9	+0.03	-3
105-120	+0.01	0	+0.01	-6	+0.03	-8	+0.03	-1
120-135	+0.02	-10	0	-2	+0.03	-2	+0.03	-7
135-150	+0.02	-4	0	+1	+0.04	-9	+0.03	-5
150-165	+0.01	0	+0.01	0	+0.03	+1	+0.03	-8
165-180	+0.01	-5	+0.01	0	+0.04	-5	+0.01	+1

¹ In 400 cc. of water.

² In 500 cc. of water.

195 minutes after ingestion of 40 gm. of urea in the series with the mouthpiece. These experiments differ from preceding studies of this nature with man in that the measurements were not made in single isolated periods but rather in an uninterrupted series of three periods with the mouthpiece and twelve periods with the helmet. The effect of preceding minor activity on the oxygen absorption is thus avoided, particularly in the series of twelve periods with the helmet apparatus.

RESPIRATORY QUOTIENT

The principal reason for this investigation was to ascertain the course of the respiratory quotient when there was a change in the alveolar carbon dioxide that was not produced by artificial means, such as inhaling high percentages of carbon dioxide or by forced overventilation, and that was unaccompanied by any metabolic oxidation process. The interpretation of the changes in the respiratory quotient for a period of 3 to 4 hours after the ingestion of a substance is dependent on the changes that would occur in the same period of time with no dose or with a control with the same volume of water as was used for the ingestion of the substance to be studied. In the series with no dose with the mouthpiece, the changes from the base-line value in $3\frac{1}{2}$ hours varied from $+0.01$ to -0.02 . Although the majority are plus in sign, the average is so small and the variations in the individual forty-four periods of the experiments are so large (-0.05 to $+0.04$) that these slight increases cannot be considered significant. In the twelve periods with the helmet following the base-line group, the changes are all positive but one, but the largest is only $+0.02$. The slight increases were caused by the almost uniform rise in the quotient in one experiment as compared with the changes in both directions in the other two experiments. With 400 and 500 cc. of water there was little change in either direction in the majority of the periods with the mouthpiece, but with the helmet in eight out of twelve periods there was a slight rise in respiratory quotient. In this series the positive changes are brought about mathematically by the almost uniform rises in one of the three experiments.

With 30 gm. of urea there was at first a slight fall in the respiratory quotient in the series with the mouthpiece. This may or may not be significant in comparison with the changes in alveolar air. One would expect that with a shift in the alveolar air toward an increasing amount of carbon dioxide there would be temporarily a lowering of the respiratory quotient. In the last two periods of the second hour there was

a slight rise of 0.02. Similarly in the third hour there was a rise of 0.02 in the third period. In the last two periods of the experiment the respiratory quotient had fallen to below the original base line. In the series with the helmet after the first three quarter-hours there was a definite rise in the respiratory quotient throughout the whole series, that is, over a period of $2\frac{1}{4}$ hours. This rise is so large, reaching in several instances 0.04, that there can be little question that there was a significant increase in the respiratory quotient. The results with the helmet were more definite because there was no interruption and therefore no omission of any variation which might take place due to the shifting from a condition of breathing through a mouthpiece to one of rest. In the series with 40 gm. with the mouthpiece, the results were somewhat similar for the first hour to those with 30 gm. of urea. However, in the second and third hours there was a definite increase in the respiratory quotient in all but one period and one cannot conclude that the increase has been entirely eliminated in the last half-hour of the experiments. With the helmet the increase on the whole was not quite so large after 40 gm. as after 30 gm. but was definite in the periods beginning 45 minutes after ingestion and ending 165 minutes after ingestion. There was thus a definite rise in the respiratory quotient after the ingestion of 30 gm. or 40 gm. of urea.

URINARY NITROGEN

In all the experiments, the urine was collected from the time the subject arrived at the laboratory until after the experiment was completed, unless the subject desired to void urine before the end of the experimental period.

For an average period of a little over 5 hours the average amount of nitrogen in the urines in seven no-dose experiments was 1.98 gm. and in eight experiments with water it was 2.26 gm. The average urinary nitrogen in experiments with 30 gm. of urea was 6.49 gm. and with 40 gm., 7.56 gm. The periods of time represented by the urea experiments were also about 5 hours. From the differences between the experiments

with and without urea it can be estimated that about 30% of the ingested urea was eliminated in the 3 to 4 hours after ingestion. In two special series of experiments, one with 500 cc. of water and the other with 40 gm. of urea in 500 cc. of water, the urine was collected every $\frac{1}{2}$ hour for 4 hours after the ingestion of the dose. The subject in these series was also given 200 cc. of water at each half-hour interval. In these urine samples, total nitrogen, urea, and ammonia were determined by the methods of Folin ('34) and associates. The ammonia-nitrogen in the control series in the 4 hours was 153, 93, and 141 mg. and each experiment did not show a wide variation in the eight half-hour periods. The amounts averaged 6.8, 3.0, and 5.3% of the total nitrogen, respectively. In the three experiments with 40 gm. of urea the total ammonia-nitrogen was 112, 97, and 98 mg. and averaged 1.4, 1.2, and 1.1% of the total nitrogen. There was thus a slightly lower ammonia elimination after the ingestion of urea. The urea-nitrogen in the water series was 1.84, 2.54, and 1.98 gm. and constituted 82, 83, and 75% of the total nitrogen. In the three experiments with 40 gm. of urea, the urea-nitrogen was 7.46, 7.48, and 7.54 gm. and formed 92, 91, and 88% of the total nitrogen. The maximum elimination of urea-nitrogen was in the second $\frac{1}{2}$ hour after ingestion and was 1.48, 1.39, and 1.36 gm. This was the period of maximum change in the alveolar carbon dioxide. The average of the urea-nitrogen in the three water experiments was 2.12 gm. and in the three urea experiments, 7.49 gm. The difference between the two series was 5.37 gm., which corresponds to about 12 gm. of urea. The total nitrogen eliminated in the three water experiments was 2.25, 3.07 and 2.64 gm. In the urea experiments the total nitrogen elimination was 8.10, 8.20, and 8.60 gm. The averages of the two series are 2.65 and 8.30 gm., respectively. The difference between the averages is 5.65, corresponding to 12 gm. of urea. Of the 40 gm. of urea ingested there was about 30% eliminated in the urine in approximately 4 hours.

DISCUSSION

In the digestion of protein and subsequent metabolism, urea is formed in quantities varying according to the rate at which the various processes take place. One would expect, therefore, that after the ingestion of meat there would be sometime during its metabolism a considerable increase in urea in the blood. Therefore, one would look for a change in either the alveolar carbon dioxide or the carbon-dioxide content of the blood during the metabolism after ingestion of protein. Hasselbalch ('12) found a lowering of the alveolar carbon dioxide after administering a diet of meat and fat for several days in contrast to the increase noted with an alkaline vegetarian diet. Bischoff and co-workers ('34) found on two occasions that after the ingestion of a pound of steak there was a fall of 3 m. Mol. in the plasma bicarbonate at the eighth hour after ingestion. Wright ('36) states that a meat diet gives rise to an excess of acid radicals, especially H_3PO_4 and H_2SO_4 derived from the oxidation of phosphorus and sulphur of the protein, and the alveolar carbon dioxide is found to fall. However, in all these cases it is probable that these effects are not the immediate results of ingestion of protein but rather ones which come on in the final stages of the formation of the end products of oxidation. Chanutin ('21) found that after 940, 810, and 1000 gm. of meat ingested by a dog there was a marked rise in the carbon-dioxide-combining power of the blood even in the first hour and states that it is possible that the interpretation of Erdt ('15) was correct in that the rise in the carbon-dioxide tension following the meal is due to the secretion of hydrochloric acid by the gastric juices. Aub and Du Bois ('17) found with two of four subjects in periods beginning $1\frac{1}{2}$ to 2 hours after ingestion of approximately 600 gm. of beef, respiratory quotients as high as 0.87 to 0.91. The other two subjects and all subjects in the basal condition gave respiratory quotients ranging from 0.77 to 0.86. McClellan, Spencer, Falk and Du Bois ('28), however, found respiratory quotients that averaged as a whole 0.75 when the subjects were given meat meals. Their basal quotients ranged

between 0.72 and 0.78, and the average was 0.76. Thus there was no effect of the ingestion of meat upon the respiratory quotient. These subjects, however, had been existing on either a low carbohydrate diet such that mild acidosis was produced or they were near the borderline for the condition to produce acidosis. Porges, Leimdörfer, and Markovici ('11) found a rise in the carbon-dioxide tension in the blood after meals and ascribed it to the gastric secretion. Since that time there have been many studies on the changes in alveolar carbon dioxide, carbon-dioxide content of the blood, and the alkaline tide in relation to the ingestion of food and the gastric secretion. The general opinion is that there is a relationship between the gastric secretion involving the formation of hydrochloric acid and the other factors mentioned. Apperly ('36), in fact, has recently come to the general conclusion that the alkaline reserve of the blood and the gastric secretion among different individuals are related. It would, therefore, appear that not only may there be a change in the alveolar air as a result of the presence of urea in the blood after the ingestion of protein but also a change in the alveolar carbon dioxide in the early part of the period of time after ingestion of protein due to gastric secretion. In the experiments here reported, when there was a change of alveolar carbon dioxide which was brought about in metabolic processes there was also an effect upon the respiratory quotient so that for a period of time the quotient was not actually as high as it should have been, and subsequently it rose, due to the release of the carbon dioxide in the blood and the return of the alveolar carbon dioxide to the pre-ingestion level. The changes in the alveolar carbon dioxide and the subsequent changes in the respiratory quotient must have been due to the presence of urea in the blood itself. Therefore, there would be no reason to infer that such a change did not take place in the normal formation of urea and its release into the blood during the process of metabolism of protein. It must be recognized that the respiratory quotient after the ingestion of protein for a long period of time does come to the normal empirical quotient

which has been used for many years, but in any interpretation of the periodic changes that take place after the ingestion of protein or various fragments of protein, it must be recognized that the changes that may occur are influenced somewhat by the temporary disturbance of the carbon-dioxide content of the blood, with the subsequent release of carbon dioxide to the outcoming air.

SUMMARY

The respiratory exchange of a human subject was determined for 3 or $3\frac{1}{2}$ hours after ingestion of 400 and 500 cc. of water or 30 or 40 gm. of urea in 400 and 500 cc. of water, respectively. Two methods were used for the determination of the respiratory exchange, 1) an open circuit apparatus with mouthpiece, and 2) an open circuit apparatus with helmet. When the apparatus with the mouthpiece was used, simultaneous determinations of the alveolar carbon dioxide were made every 7 to 8 minutes. There was no effect upon the total metabolism as a result of the ingestion of 30 or 40 gm. of urea when compared with the measurements for the same periods of time in no-dose experiments and those with water. In the experiments with water there was a slight drop in the alveolar carbon dioxide for most of the $3\frac{1}{2}$ hours after the ingestion. With 30 and 40 gm. of urea there was a marked rise in the alveolar carbon dioxide for 2 to $2\frac{1}{2}$ hours. The respiratory quotient was not changed significantly in either the no-dose experiments or the experiments with 400 and 500 cc. of water. With 30 and 40 gm. of urea there was a significant rise in the respiratory quotient beginning at the fourth or sixth quarter-hour after ingestion and lasting until the end of the third hour after ingestion. The study shows that along with an alteration in the alveolar carbon dioxide there is an alteration in the respiratory quotient as the result of the alkalosis following the ingestion of urea. As the alveolar carbon dioxide also rises due to the gastric secretion after the ingestion of protein, it is pointed out that there may be two causes for alterations in the respiratory quotient from the

true respiratory quotient after the ingestion of protein, one the gastric secretion containing hydrochloric acid and the other the urea that ultimately results as a metabolic product of the transformations of protein in the body.

The determinations of the respiratory exchange and alveolar air were made by Mr. B. James, assisted by Mr. R. E. Murray, who also made the analyses of the urines.

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THE BASAL METABOLISM IN PREGNANCY

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THREE FIGURES

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The course of the basal metabolism throughout pregnancy is well established, the largest group of observations being those of Rowe and Boyd ('32) who made serial determinations on seventy-seven women. They describe a fall in the energy requirement from a normal to a subnormal level during the third to the fourth month of gestation, the low level being reached in about 4 weeks. "From this point on, during the last 6 lunar months there is a steady increase in the basal metabolic rate amounting to 13% or more in excess of that conditioned by the gross increase in body weight."

The point has been at issue as to whether or not this rise could be explained as the heat production resulting from a new protoplasmic mass, or whether some glandular influence would not have to be invoked to explain the change. Sandiford and Wheeler ('24), analyzing the data from their single case and those in the literature, concluded that "the energy production of a unit mass of the mother's protoplasmic tissue remains unchanged throughout the course of pregnancy, and that such increases in the total heat production as occur are due to the increasing mass of active protoplasmic tissue consisting in large part of the fetal tissues and in lesser part of

maternal structures." Since Carpenter and Murlin ('11) had shown that just after birth the energy of the mother and child had shown no deflection, Sandiford and Wheeler argue that the fetus as well as the baby after birth should be regarded as a being independent of the mother and its heat production calculated on its own surface area which is proportional to its own active protoplasmic mass before and after delivery. Harding ('25) reviewed the entire subject and felt that the experiments recorded up to that time offered "no support to the idea that a special hyperactivity of the maternal thyroid occurs during pregnancy." He did note, however, that the calculations of Zuntz and of Carpenter and Murlin showed an increased metabolic rate in the mother, as distinct from the fetus, of about 4%. The method of approach taken to reach this conclusion—the calculation of the maternal and fetal areas independently and the combining of them into a so-called total effective area—is questioned by Rowe, who points out several flaws in the reasoning. First, it ignores wholly the lowering in heat production which takes place early in pregnancy. Secondly, when a large series is considered, and this total effective area is divided into the caloric output, while a fairly constant quotient is obtained, on close examination this value proves to show a definite progression defining a curvilinear relationship with a maximum approximately midway in the series. Thirdly, when curves are constructed in which there are plotted total calories, maternal calories, and the fetal areas, the data all show a linear change in basal rates but a curvilinear relationship of the fetal area, and hence a discrepancy in the attempt to compromise the two sets of data.

EXPERIMENTAL PROCEDURE

The energy data here recorded were obtained on a subject whose mineral metabolism was previously reported (Hummel et al., '37). The fasting metabolism was determined under basal conditions by the gasometer method. Gas analyses were made in duplicate or triplicate with the Haldane-Henderson apparatus, samples of approximately 9.5 cc. being used. Nitrogen balances were calculated from the analyses of food, urine,

feces, and breast milk. Calculations of the 'basal' metabolic mixture were made from the table of Lusk ('24). These are, of course, not accurate since the 24-hour urine was not subdivided and the figure used for the basal hour's nitrogen was one-twenty-fourth of the total. The error is, however, constant and we feel that the calculation brings out a point pertinent to this discussion.

FINDINGS

The energy data are recorded in table 1. Plotted graphically (fig. 1) the curve follows that of Rowe. When this curve is plotted against the cumulative nitrogen and sulfur balance a striking parallelism is suggested, which, however, is not maintained during the puerperium.

That the rise in metabolism is not incidental to an increased intake of food is evident from figure 2. While an attempt was made to have the food intake governed to a large extent by appetite and while there is a steady rise in food intake throughout the first four periods, from that point up to the delivery, the caloric intake is constant as is the nitrogen.

It is worth noting (fig. 3) that the rise in metabolism is not paralleled by an increase in urinary nitrogen, and that the metabolic mixture using one-twenty-fourth of the daily nitrogen as the basal hour shows a decline in the percentage of calories derived from protein.

COMMENT

Any attempt to interpret these findings as the relatively simple mathematical result of increased mass must ignore a number of well-established glandular changes concomitant with pregnancy and must make the assumption that these have occurred without influencing the maternal oxygen consumption. An increase in volume to the extent of two and one-half times the normal has been demonstrated in the case of the pituitary gland (Kolde, '12). The changes are almost limited to the anterior lobe. These changes are demonstrable between the third and fourth months.

TABLE 1

PERIOD	DATE	TOTAL CALCS. PER HR.	SURFACE AREA	CALCS./ SQ.M./HR.	BMR	RQ	EST. ¹ TOTAL CALCS.	BASAL CALCS./ 24 HR.	METABOLIC MIXTURE					
									P		F		O	
									gm.	% cals.	gm.	% cals.	gm.	% cals.
1	3/16	58.7	1.51	38.9	- 3	0.79	57.0	1367	3.9	28.7	3.0	48.0	3.3	23.6
2	3/25	59.3	1.52	39.0	- 2	0.78	58.1	1396	3.9	26.0	2.9	46.0	3.3	28.0
4	4/ 1	60.0	1.52	40.1	± 0	0.84	60.0	1441	3.0	29.0	2.5	31.0	5.9	40.0
5	4/ 8	58.7	1.52	38.6	- 3	0.82	57.6	1408	3.2	22.4	2.9	46.5	4.4	31.0
7	4/15	63.7	1.53	41.6	+ 4	0.81	62.5	1528	3.1	20.4	3.7	53.0	4.1	26.6
8	4/22	60.9	1.53	39.8	± 0	0.81	59.6	1461	3.4	23.2	3.1	47.6	4.2	29.2
9	4/29	62.0	1.53	40.3	+ 1	0.80	61.1	1488	3.0	20.3	3.6	55.0	3.7	25.0
11	5/ 6	62.5	1.57	39.8	± 0	0.83	61.1	1499	3.3	21.9	2.6	40.0	5.7	38.1
12	5/13	65.8	1.56	42.2	+ 5	0.80	64.8	1579	3.0	19.3	4.0	56.2	3.9	24.5
14	5/20	69.5	1.57	44.6	+11	0.77	68.9	1668	2.9	17.2	5.0	66.8	2.7	16.0
17	6/ 5	52.8	1.45	36.4	- 4	0.81	51.8	1267	3.3	26.0	2.5	45.2	3.6	28.4
18	6/11	51.1	1.48	34.5	- 9	0.83	50.0	1226	3.0	24.8	2.2	40.2	4.2	35.0
19	6/17	50.8	1.46	34.8	- 8	0.81	49.9	1219	3.0	24.0	2.5	46.0	3.6	30.0
20	6/24	50.2	1.45	35.3	- 7	0.76	50.2	1204	3.0	24.1	3.7	67.6	1.0	8.3
22	7/ 2	49.4	1.45	34.1	-10	0.77	48.3	1183	3.0	25.2	3.5	66.7	0.9	8.1
23	7/ 8	45.0	1.46	30.8	-19	0.80	44.1	1080	2.8	26.5	2.4	50.3	2.5	23.2
24	7/15	48.1	1.47	32.4	-13	0.81	47.0	1154	3.1	27.0	2.7	53.0	2.3	20.0
26	7/22	51.5	1.48	34.8	- 8	0.80	50.5	1236	3.1	25.3	2.9	51.9	2.8	22.8

¹ Boothby Sandiford standards.

Gaebler ('35) has shown that the growth hormone from the anterior lobe of the pituitary, administered to dogs, produced a marked increase in heat production and a sharp increase in

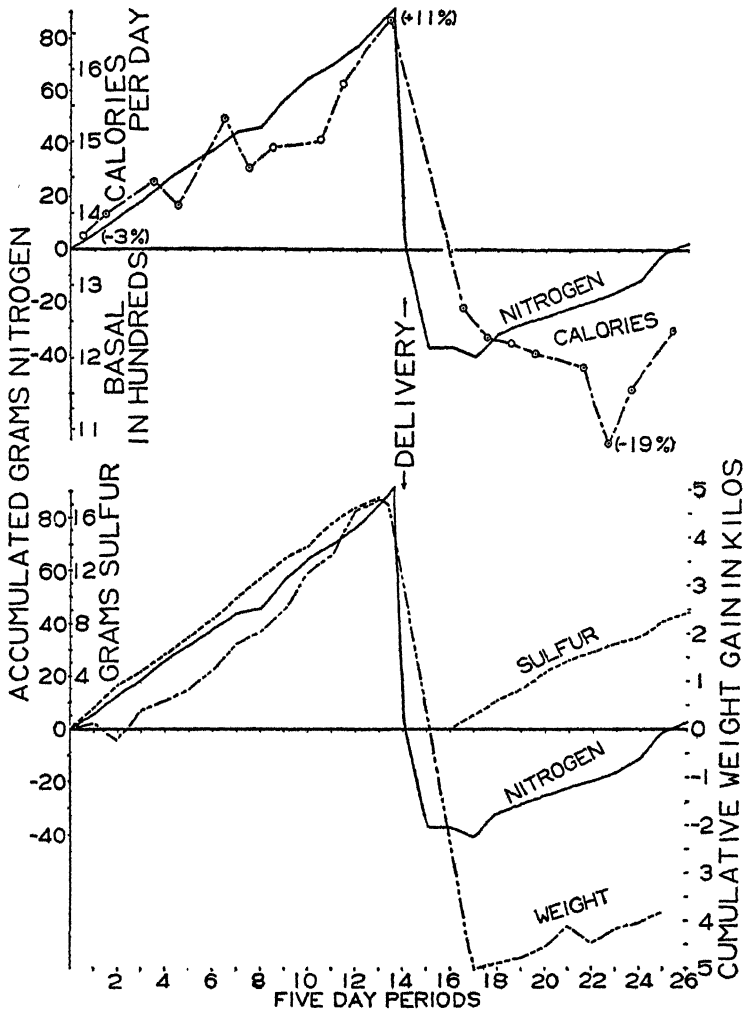


Figure 1

nitrogen retention. Similar changes were noted in thyro-parathyroidectomized dogs. Changes simulating these qualitatively, but less marked quantitatively and delayed in their

appearance, were noted with pituitary-like substance from pregnancy urine.

Evidence for a change in the thyroid during pregnancy is definite, though somewhat less striking than in the case of the pituitary, and whether the change is one of true hypertrophy and hyperplasia or merely transient is still under discussion.

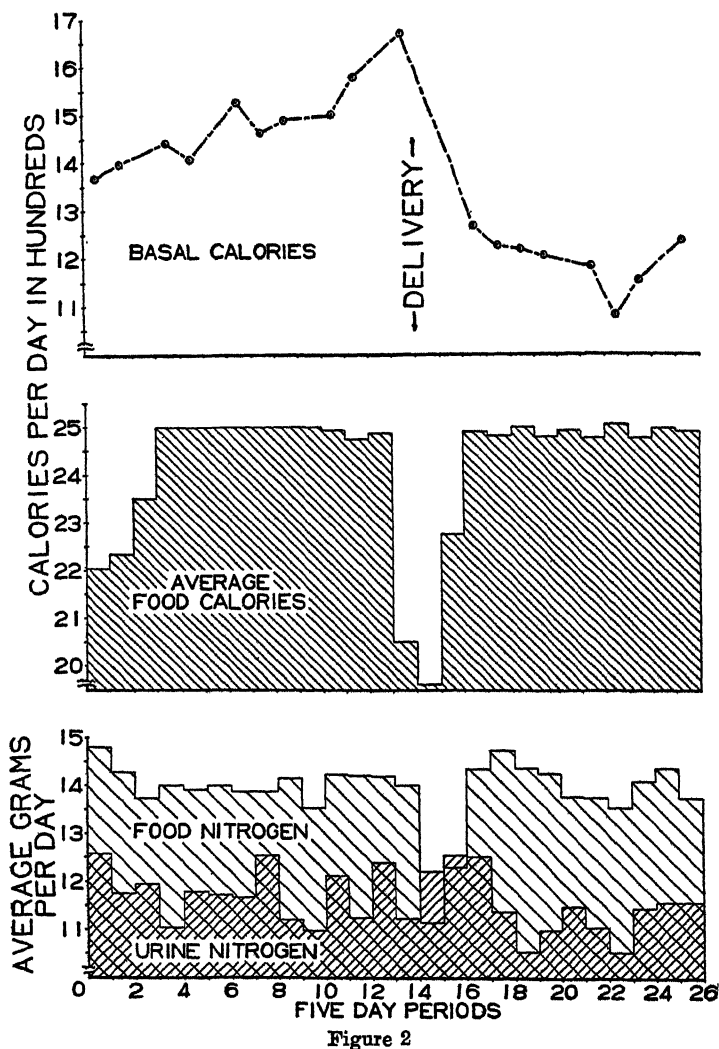


Figure 2

The demonstration of the steady increase in blood iodine from the second to the tenth months and a drop in the first 2 weeks of the puerperium (Borkelmann and Scheringer, '30) together

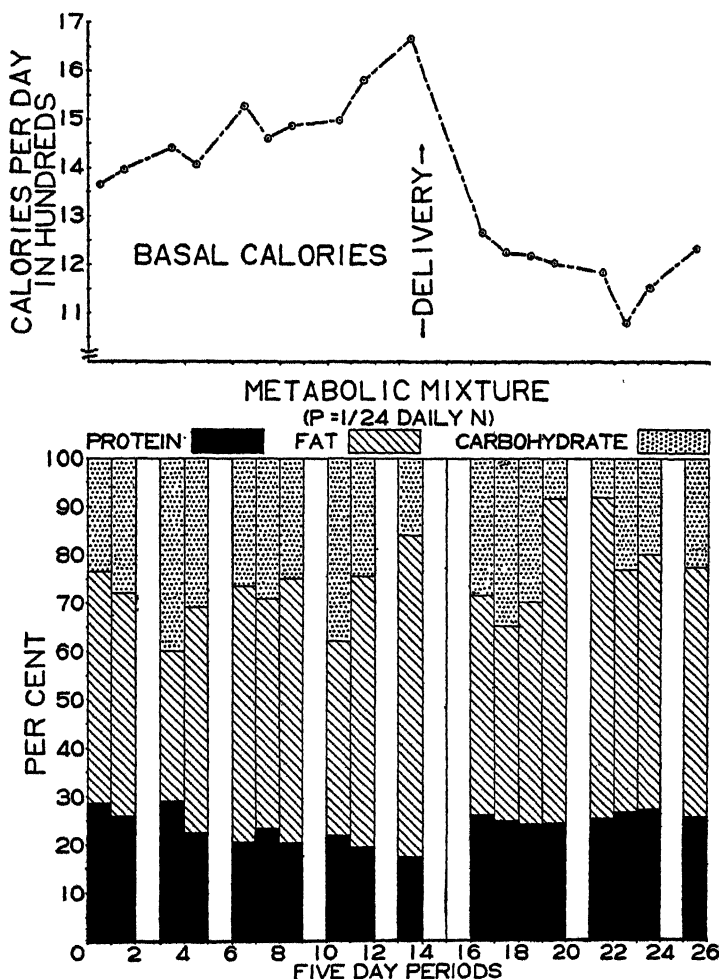


Figure 3

with the demonstration of a glycogen diminishing substance in the blood of pregnant women (Eufinger et al., '29) suggest an increase in thyroid function.

In studying the data here presented the changes noted are very suggestive of those observed by Gaebler. We have calculated what the metabolic mixture would be if the basal hour's

TABLE 2a

Continuous daily¹ nitrogen balances during pregnancy. Subject—I.M.B.

PERIOD	DAYS OF PREGNANCY	WEIGHT OF SUBJECT	NITROGEN INTAKE	NITROGEN OUTGO		NITROGEN BALANCE
				Urine	Feces	
		<i>kg.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
I	214-218	52.0	14.82	12.46	1.18	+1.18
II	219-223	52.1	14.28	11.72	1.09	+1.47
III	224-229	52.3	13.67	11.92	0.98	+0.77
IV	230-233	52.6	14.02	10.96	1.10	+1.96
V	234-238	52.8	13.96	11.80	0.95	+1.21
VI	239-243	53.0	13.99	11.75	1.04	+1.20
VII	244-248	53.5	13.85	11.62	0.95	+1.28
VIII	249-253	54.1	13.86	12.53	1.01	+0.32
IX	254-258	54.4	14.13	11.21	0.70	+2.22
X	259-263	54.9	13.51	10.95	1.02	+1.54
XI	264-269	55.6	14.20	12.16	1.20	+0.84
XII	270-273	56.0	14.19	11.29	1.15	+1.75
XIII	272-276	56.7	14.17	12.38	1.08	+1.79
XIV	277		14.69	11.05	0.35	+3.29
	278		14.36	10.17	...	+4.19
	279		13.04	11.31	0.84	+0.89
	280 ¹		8.62	0.84	-10.15
	Mean		13.84			1.21
	Total storage					+83.77 ²
	Day of delivery		9.19	Urine	3.78 gm.	
				Feces	0.84	
				Blood	15.58	
				Placenta	15.48	
				Amniotic fluid	0.35	
					36.03 gm.	
				Balance for day	-26.84 gm.	

¹ Vomitus 0.69 gm. N.

² Total accumulation found by the sum of the product of the daily storage and the number of days in each period.

³ Mean of 5-day period.

urine corresponded to one-twenty-fourth of the total urine. This shows that the rising metabolic rate is accompanied by a relative fall in calories derived from protein and a relative

increase from the calories derived from fat. The urine nitrogen is relatively constant showing a very slight tendency to decline.

TABLE 2b

Continuous daily¹ nitrogen balances during puerperium. Subject—I.M.B.

DAY POSTPARTUM	WEIGHT OF SUBJECT	NITROGEN INTAKE	NITROGEN OUTGO					
			Urine	Feces	Breast milk	Blood	Vomit	Balance
	kg.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	...	11.13	16.95	0.84	...	0.34	...	— 7.00
2	...	11.06	12.08	0.35	...	1.22	...	— 2.59
3	...	12.26	10.15	1.38	...	+ 0.73
4	...	9.26	7.25	0.34	0.29	0.94	...	+ 0.44
5	...	11.91	16.80	...	0.29	1.34	1.19	— 7.71
6	...	10.21	10.84	0.35	0.29	0.74	1.42	— 3.43
7	...	11.50	13.93	2.17	0.33	0.89	...	— 5.81
8	...	11.39	18.48	1.87	0.33	1.15	...	—10.34
9	...	12.41	7.86	1.25	0.33	0.46	...	+ 2.51
10	...	13.47	12.95	0.75	0.33	0.53	...	— 1.09
11	...	14.72	11.25	0.45	0.33	0.72	...	+ 2.13
Mean		11.76						— 2.92
Total								—32.16

PERIOD	LACTATION							
XVII	12-16	47.0	14.30	12.40	1.43	0.68	0.35	— 0.56
XVIII	17-22	47.3	14.74	11.17	1.67	0.52	0.21	+ 1.17
XIX	23-26	47.5	14.34	10.55	2.14	0.32	0.10	+ 1.23
XX	27-31	47.6	14.22	10.98	1.43	0.98	...	+ 0.83
XXI	32-37	47.6	13.79	11.51	1.53	1.09	...	— 0.34
XXII	38-42	47.6	13.72	11.06	1.23	1.08	...	+ 0.35
XXIII	43-47	47.9	13.51	10.54	1.68	0.50	...	+ 0.79
XXIV	48-52	48.0	14.10	11.52	1.12	0.17	...	+ 1.29
XXV	53-58	48.2	14.36	11.59	1.37	0.03	...	+ 1.37
XXVI	59-62	...	13.73	11.59	1.46	...	0.15	+ 0.53
Mean			14.38					+ 0.67
Total								+33.74

¹ Mean of 5-day period.

It is our feeling that the data here presented favor the view that the rise in the metabolism in pregnancy is to a large extent explained on the basis of an increase in active protoplasmic mass; but that the behavior of the nitrogen excretion and the composition of the metabolic mixture—a relative fall in the protein with a rise in fat—taken in conjunction with

the known facts regarding the changes in the anterior lobe of the pituitary gland, make it seem reasonable to include a hyperactivity of this gland in any explanation that would seem complete.

TABLE 3

Accumulative storage of nitrogen and sulfur with change in body weight during pregnancy, puerperium and lactation

PERIOD	ANTE PARTUM	N	S	CHANGE IN BODY WEIGHT
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
I	70-66	5.9	1.6	+ 114
II	65-61	13.3	3.3	- 226
III	60-55	17.9	4.3	+ 341
IV	54-51	25.7	5.6	+ 565
V	50-46	31.8	6.9	+ 581
VI	45-41	37.8	8.4	+1200
VII	40-36	44.7	9.9	+1785
VIII	35-31	46.3	11.3	+2019
IX	30-26	57.4	12.9	+2474
X	25-21	65.1	13.9	+3229
XI	20-15	70.1	15.6	+3613
XII	14-11	77.1	16.4	+4547
XIII	10- 6	86.1	17.5	+4786
XIV	5	84.3		+4666
Total accumulation		84.3	17.5	+4666
Loss during delivery		26.8		
Estimated fetal * composition		58.6		
Puerperium 11 days		-32.2		
Total loss		117.6		9677
PERIOD	POST PARTUM	N	S	CHANGE IN BODY WEIGHT
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
XVII	12-16	-36.1	1.0	-5011
XVIII	17-22	-29.1	2.2	-4884
XIX	23-26	-24.2	3.0	-4768
XX	27-31	-20.0	4.3	-4531
XXI	32-37	-22.1	5.1	-4110
XXII	38-42	-20.3	5.9	-4478
XXIII	43-47	-16.4	6.6	-4180
XXIV	48-52	- 9.9	7.4	-4019
XXV	53-58	- 1.7	8.3	-3809
XXVI	59-62	+ 0.4	8.8	

SUMMARY AND CONCLUSIONS

Data are recorded on the basal metabolism of a young primipara whose mineral balance was determined concurrently. They are interpreted as favoring the view that the changes, while to a large extent understandable as a result of the formation of a new protoplasmic mass, as evidenced by the parallelism between the cumulative nitrogen and sulfur balances and the basal heat production, are at the same time under a hormonal influence contributing to this same synthesis.

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THE ANTIRACHITIC ACTIVITY OF VARIOUS FORMS OF VITAMIN D IN THE CHICK

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ONE FIGURE

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Early in 1930, Hess and Supplee reported that a supplement of irradiated ergosterol equivalent to many times the protective dose of cod liver oil failed to promote normal calcification in the chick. Shortly thereafter, papers by Massengale and Nussmeier ('30) and Mussehl and Ackerson ('30) also indicated that irradiated ergosterol was much less effective in preventing leg weakness in chicks when the potency of the preparations was judged by the usual rat assay technic.

Following the initial demonstration of the species difference in response to the different forms of vitamin D, attempts were made to determine the ratio of antirachitic effectiveness (rat to chick) of irradiated ergosterol to cod liver oil. In the hands of different observers the results have been as follows: Massengale and Nussmeier ('30) 1:100; Steenbock, Kletzien and Halpin ('32) 1:40 to 1:100; Bethke et al. ('33) 1:15 to 1:20; Russell et al. ('32) 1:144 to 1:192; Massengale and Bills ('36) 1:50 to 1:100 or more; Bills et al. ('37) 1:31 to 1:100; Haman and Steenbock ('36) 1:20; Grab ('36) 1:400 in spring, 1:100 to 1:400 in fall or winter and 1:24 to 1:32 in summer.

Various other forms of vitamin D have also been studied from this point of view. Irradiated crude cholesterol has been shown by Waddell ('34) to be equivalent to cod liver oil and superior to irradiated ergosterol in preventing the development of leg weakness in chicks. The antirachitic value of a

semi-amorphous preparation of irradiated 7-dehydrocholesterol for the chick has been reported by Grab ('36) to be one-half to one-third as great in the spring, one-fifth in the summer and one-thirteenth as effective in early summer as for the rat. Koch and Koch ('36) tested on chicks a series of sterol derivatives including irradiated 7-dehydrocholesterol prepared by the method of Windaus, Lettre and Schenck ('35). On the basis of the weight gain, bone ash and appearance, the chicks receiving 10 rat units of irradiated 7-dehydrocholesterol per 100 gm. of diet were more completely protected against leg weakness than those receiving 10 rat units of cod liver oil per 100 gm. of diet. A further experiment revealed that 35 rat units of irradiated corn oil phytosterol were fully equivalent for chicks to 100 rat units of irradiated ergosterol but inferior to 10 rat units of cod liver oil. Bills, Massengale, Imboden and Hall ('37) observed, on the basis of the bone ash values found, that the antirachitic efficiency for the chicken of irradiated 7-dehydrocholesterol, irradiated cholesterol from spinal cord and cod liver oil differed in no significant degree. McDonald ('36) found that rat unit for rat unit, the effectiveness of irradiated 22-dihydroergosterol for the chick more closely approximated that of cod liver oil than that of irradiated ergosterol. Boer, Roerink, van Wijk and van Niekerk ('36), working with a special cholesterol, isolated the natural chicken pro-vitamin. Chemical and biological tests indicated that it was 7-dehydrocholesterol.

Massengale and Bills ('36) reported that the ratio of the antirachitic efficiency of cod liver oil to irradiated ergosterol in the chick for the production of bone ashes amounting to 40% was 1:31, but as the bone ash increased to 47.3%, the ratio increased to 1:220. Thus it appears that the actions of cod liver oil and irradiated ergosterol in the chick are strictly comparable only under specified conditions.

Due to the rather unsatisfactory agreement of the reports in the literature and the fact that many of the preparations which have been tested were relatively impure, it seemed worth while to make a comparative study of the effectiveness

of different types of purified vitamin D preparations in the rat and chick. In this study we have included the pure crystalline forms of irradiated ergosterol, irradiated 7-dehydrocholesterol, a standard cod liver oil, an irradiated cholesterol and Viosterol (A.R.P.I. process) in oil.

OUTLINE OF TEST METHODS

All products, which were tested for their prophylactic values against rachitis in chickens, were assayed in triplicate, using the U.S.P. antirachitic (curative) vitamin D test method on rats.

White Leghorn chicks of a standard breed were received in the laboratory on February 2, 1937, on the second day of life. All chicks were placed in individual wire cages containing water cups. Feeding was begun on the third day of life and continued for 28 successive days thereafter. On institution of feeding, the birds were segregated into twenty-four groups of fifteen to twenty-five chicks each.

The diet fed was a modification of that described by Hart, Kline and Keenan ('31) and consisted of ground yellow corn (57%), wheat flour middlings (25%), crude domestic casein (12%), precipitated calcium carbonate (1%), calcium phosphate (1%), dried baker's yeast (1%), and sodium chloride (1%). The final 2% consisted of cod liver oil (group 24 positive controls), of corn oil alone (group 23 negative controls), or corn oil in which the vitamin D supplement was dissolved. The diet was mixed freshly each third day of the test. The vitamin D supplement was adjusted at such times on the basis of predetermined normal food intakes for this stock breed in order to assure an accurate day dosage of each of the medicaments.

The observations taken during the 28-day period of study included food intake and body weights at 3-day intervals. All birds were sacrificed on the thirtieth day of life. The two tibiae were cleansed of soft tissues and labeled. One was fixed in 90% ethyl alcohol for microscopic examination and

for a permanent photographic record of the degree of calcification present. Microscopic observations included measurements of the width of the metaphyses (standardized micrometer scale) and an examination of serial sections of the bones. The latter test, which will be described elsewhere, permits a rapid evaluation of the degree and character of the calcification present, comparable to that of the standard U.S.P. antirachitic line test. The second bone of each chick was dried, crushed, wrapped in a piece of filter paper and extracted in a Soxhlet extractor for 48 hours with alcohol and an equal period with ether. The bones were then dried at 105°C. to constant weight. They were then ashed for 4 hours at 1000°C. and again weighed.

DESCRIPTION OF THE VITAMIN D PREPARATIONS TESTED

An ample volume of cod liver oil for completion of all tests was standardized on rats, using the U.S.P. antirachitic vitamin D test methods with the U.S.P. reference oil. This product, which assayed 100 units per gram, was also checked with the international vitamin D standard.

The crystalline irradiated ergosterol (vitamin D₂) used was prepared by the method of Windaus, Linsert et al. ('32). The product had a formula of C₂₈H₄₄O, a melting point of 116° to 117°C., and maximum absorption spectroscopically at 265 mμ. The specific rotation of the crystals in acetone dissolved in 1, 3-propanediol was $(\alpha)_{\text{D}}^{20} = +81.5^\circ$. Vitamin D₂ is insoluble in water but soluble in organic fat solvents.

The crystalline irradiated 7-dehydrocholesterol (vitamin D₃) used was prepared by the method of Windaus, Lettre and Schenck ('35). This product had a formula of C₂₇H₄₄O, a melting point of 82° to 84°C. Its absorption maximum was at 265 mμ and the specific rotation in acetone was $(\alpha)_{\text{D}}^{20} = +83^\circ$. The crystals are practically insoluble in water but soluble in organic fat solvents.

The crystalline vitamins D₂ and D₃ and their physical constants were furnished through the courtesy of Dr. O. W. Barlow of the Winthrop Chemical Company, Rensselaer, New York.

The irradiated crude spinal cord cholesterol in oil was furnished by Dr. J. Waddell of the Acetol Products, Incorporated, of New Brunswick, New Jersey. The potency of the product (lot no. 4499) was 10,000 U.S.P. vitamin D units per gram.

The Viosterol (A.R.P.I. process) in oil was a vitamin D concentrate with a potency of 1,000,000 U.S.P. units per gram in sesame oil. This preparation was described as "a solution in oil of ergosterol activated by the action of low speed electrons produced by suitable controlled electrical means." (Am. Med. Assoc. Council on Pharmacy and Chemistry, '37). The product was furnished through the courtesy of the Research Laboratories of the American Research Products, Incorporated, of Minneapolis, Minnesota.

RESULTS

On the basis of the U.S.P. antirachitic vitamin D test, crystalline vitamin D₂ assayed 0.025 microgram per unit, or 40,000,000 units per gram. Crystalline vitamin D₃ under similar conditions corresponded exactly in antirachitic potency to vitamin D₂. The spring and early summer day dose of the semi-amorphous powdered product obtained from irradiated 7-dehydrocholesterol was reported by Grab ('36) to be 0.06 γ or approximately 17,000,000 units per gram. A product similar to that tested by Doctor Grab, obtained from Prof. H. Hoerlein of the Pharmaceutical Division of the I. G. Farbenindustrie, had a vitamin D potency, according to our assays, of 24,000,000 units per gram. The vitamin D₃ tested in these studies was the purified crystalline product.

The results of our assays of several vitamin D preparations by the prophylactic test on chickens are illustrated in table 1. Under the experimental conditions, a femur ash of 45% or more may be considered normal. The amount of vitamin D necessary to produce normal ash values in chicken bones would appear to be as follows: crystalline vitamin D₃ 2.4 I.U. (international unit), cod liver oil 2.2 I.U., irradiated cholesterol 2.7 I.U., vitamin D₂ 120 I.U., and Viosterol (A.R.P.I.

TABLE 1
Prophylactic study with vitamin D in rachitic White Leghorn chicks

GROUP NO.	SOURCES OF VITAMIN D	DAILY DOSE		TOTAL FOOD INTAKE AVERAGE	WEIGHT		METAPHYSIS		INDEX OF CALCIFICATION AVERAGE	BONE ASH AVERAGE
		Expected median	Actual median		Original median	Gain median	Width average	Per cent of positive control		
		I.U.	I.U.	gm.	gm.	%	mm.			%
1	Cod liver oil	1	0.98	474.5	35.5	410	1.125	168	1.9+	41.17
2		2	2.175	492.5	35.5	484	0.71	106	2.5	45.01
3		3	3.225	504.5	36.0	448	0.72	107.4	3.0	45.57
4		6	6.68	541.0	35.0	529	0.62	92.6	3.05	47.05
5	Crystalline vitamin D ₂	1	1.0	492.5	34.0	402	0.885	132	1.9	42.26
6		2	2.38	538.0	36.0	545	0.67	100	2.62	44.96
7		3	3.37	514.5	34.0	505	0.67	100	3.25	45.98
8		6	6.06	546.0	35.0	517	0.60	89.5	3.5	46.52
9	Irradiated cholesterol	1	0.995	453.0	36.0	349	1.61	240	1.5	39.15
10		2	2.22	523.0	35.0	509	1.005	149.4	2.5	42.70
11		3	3.16	509.0	35.0	472	0.71	106	3.15	46.56
12		6	6.37	507.5	38.0	479	0.71	106	3.25	45.52
13	Crystalline vitamin D ₂	10	9.35	387.0	36.0	289	3.20	478	1.0	32.34
14		20	20.3	414.6	35.0	367	2.57	384	1.0	38.13
15		50	50.3	431.0	35.0	441	0.967	144	2.38	42.34
16		100	102.5	506.5	35.5	462	0.745	111.2	3.25	44.81
17	Viosterol (A.R.P.I. process)	200	206.0	506.5	36.0	445	0.73	109	3.25	46.38
18		10	10.1	463.5	33.0	344	2.285	342	1.0	33.40
19		20	19.85	457.0	35.0	375	2.29	342	1.76	36.56
20		50	51.0	497.0	35.0	403	1.89	282	2.0	40.83
21	Negative control—2% C.O.	100	106.4	517.5	35.5	412	0.70	104.2	3.0	44.55
22		200	210.0	510.0	34.5	451	0.70	104.2	3.0	45.38
23	Positive control—2% C.L.O.		0	305.9		284	4.18	—625	—2.0	29.45
24			36.3	492.2		468	0.67	100	+3.5	46.12

process) 150 I.U. The efficiency ratio of cod liver oil to Viosterol (A.R.P.I. process), rat unit for rat unit, in the chick is 1:63. This value is between that reported by Russell, Taylor and Wilcox ('34) and that of Bethke, Record and Kennard ('33). These groups of workers based their comparison on the minimum protective dose. Massengale and Bills ('36) consider a bone containing 46 to 47% of ash as normal according to their technic. For bones containing 46% ash they find the ratio of cod liver oil to irradiated ergosterol to be 1:74 which is in good agreement with the present results.

The results obtained in these experiments are not as clear cut as might be desired. It will be noted that when 1 unit a day of cod liver oil, vitamin D₃ or irradiated cholesterol was fed, the order of efficiency, according to the bone ash, from high to low, was vitamin D₃, cod liver oil and irradiated cholesterol. When two units were fed, vitamin D₃ and cod liver oil were equal in potency and superior to irradiated cholesterol. At the three unit level, vitamin D₃ and cod liver oil are again equal, but irradiated cholesterol was questionably superior. At the highest dose fed, the order then became cod liver oil, vitamin D₃ and irradiated cholesterol, but it is doubtful whether the differences noted have any significance since the ash values of all bones were within normal limits. However, the results do reveal that the bone ash is not entirely satisfactory for the estimation of the potency of vitamin D preparations in the chicken. Similar criticisms may be made of the use of gain in weight, and, to a lesser extent, of the metaphysis width and direct examination of sections of the bone (modified line test), when used individually as a basis for vitamin D assay in the chicken. It would appear that the use of all four types of data when evaluating the results of vitamin D administration in the chicken would contribute to the accuracy of assays. In the final estimation of the curative day dose, the experimental results were compared with those of the positive control group as to gain in body weight, as well as the width of the metaphysis. Bones with a 3+ index

of calcification, as indicated by a modified form of the anti-rachitic line test, and 45% or more ash were considered normal. On this basis, the minimal protective dose for chickens appears to be 2.5 I.U. of cod liver oil, 2.5 I.U. of vitamin D₃, 2.7 I.U. of irradiated cholesterol, 85 I.U. of vitamin D₂ and 120 I.U. of Viosterol (A.R.P.I. process). The antirachitic efficiency ratio of cod liver oil or crystalline vitamin D₃ to crystalline vitamin D₂ in the chick then becomes 1:34. The corresponding values for Viosterol (A.R.P.I. process) are 1:48. These ratios are lower than those based on bone ash values alone. These results indicate that cod liver oil, irradiated cholesterol and vitamin D₃ differ to no significant degree as to potency in the chick, rat unit for rat unit. It is probable that at least one of the pro-vitamins found with cholesterol is 7-dehydrocholesterol (pro-vitamin D₃), as has been demonstrated in a special case by Boer et al. ('36). Hence it is not surprising that nearly identical results were obtained with irradiated cholesterol and vitamin D₃. These results, however, cannot necessarily be used as proof that 7-dehydrocholesterol is the only active principle in cholesterol or cod liver oil since it is possible for two chemical compounds to be present simultaneously, each of which have the same antirachitic activity in the chick as has already been shown to be true in the rat.

Crystalline vitamin D₂ on the basis of these data appears to be somewhat more effective in the chicken than the Viosterol (A.R.P.I. process) tested. This observation is in line with the fact that Viosterol is a mixture of compounds, the most important of which is vitamin D₂.

Figure 1 illustrates the average effects of various dosage levels of the several types of vitamin D upon the bone ash. The experimental points fall about two curves which are dissimilar in nature. For convenience in graphing, the abscissa has been multiplied by twenty for the Viosterol (A.R.P.I. process) and vitamin D₂ data.

The response curves for these two groups of compounds, as illustrated by those of vitamin D₂ and D₃ in the chick are

essentially different and cannot be made to coincide by expansion or contraction of the coordinates. These data, although not directly comparable to those of Massengale and Bills ('36), because of differences in technic, confirm the observations of these authors, relative to the divergent results obtained by different investigators, when comparing

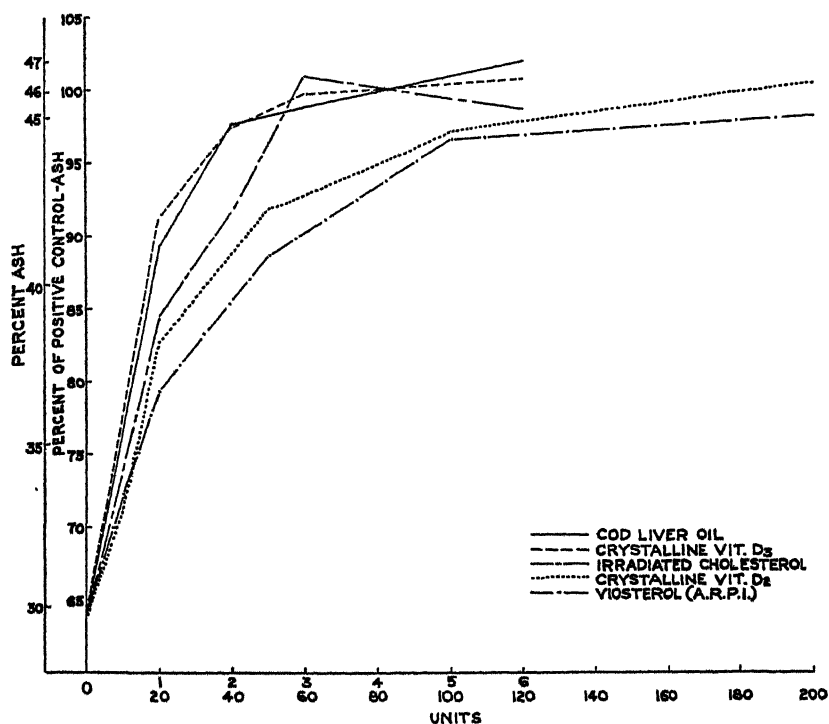


Fig. 1 Response of bone ash to various doses of vitamin D. The abscissa for vitamin D₂ and Viosterol (A.R.P.I. process) is twenty times that of the other substances.

the efficiency of cod liver oil with that of irradiated ergosterol, rat unit for rat unit on chickens. When comparisons of these substances are to be made in chickens, the degree of calcification of the bone must be considered. No comparison can safely be made when the bones have different ash values.

Strictly speaking, these two groups of substances cannot be compared for their antirachitic efficiency in the chick since they apparently function in a slightly different manner physiologically. It may be suggested that irradiated 7-dehydrocholesterol, cod liver oil and irradiated cholesterol can be readily utilized by the organism, while the utilization or conversion of Viosterol (A.R.P.I. process) or vitamin D₂ into a substance suitable for metabolism in the chicken is relatively slow or incomplete. In the rat it appears that these changes can be readily made since vitamin D₂ and D₃ have the same potency per gram.

Russell et al. ('34) found, after medication with cod liver oil, that only relatively small amounts of vitamin D are stored in the liver of the chicken, while much larger amounts are stored when irradiated ergosterol was fed. The assays of the chicken livers were made by means of the usual rat technic. Since the rat will respond equally well to the vitamin in cod liver oil and to irradiated ergosterol, it is not possible to state with certainty the form in which the vitamin was stored in the livers of the chicken. These authors have suggested that more than one vitamin D is present in cod liver oil and that a form is present in the livers of the chicks fed cod liver oil to which the chick responds but which is not effective in the rat. Bills et al. ('37) have concluded that more than one form of vitamin D exists in fish liver oils. However, commercial cod liver oils which may consist of oil from the livers of *Gadus morrhua* and other species of the family Gadidae have the same efficiency in the rat and in the chicken. It is not improbable that the storage noted in chicken livers after irradiated ergosterol feeding was in the form of vitamin D₂. The degree of utilization or the rate of conversion by the chicken of this compound into a physiologically more active form of vitamin D may then be the controlling factor in the prophylactic or antirachitic effects of irradiated ergosterol in this species.

Bethke et al. ('33) and Russell et al. ('34) have suggested that two or more factors may be present in irradiated

ergosterol, one of which is effective in both the chicken and the rat and the other only in the rat. In the interpretation of the present experiments, this argument does not hold since both the crystalline vitamin D₂ and Viosterol (A.R.P.I. process) were found to have relatively the same potency when tested on rats, as well as on chicks. Bills, Massengale, McDonald and Wirick ('35) also found after assaying seven specimens of irradiated ergosterol on rats, that when the species efficiency ratio was taken into consideration, the anti-rachitic values of the several products on chickens were identical. Thus it does not appear that the lower antirachitic efficiency of irradiated ergosterol for the chick as compared with the rat is influenced significantly by the contaminants to be found in the various irradiated ergosterol preparations, although these data suggest that the chick utilized crystalline vitamin D₂ more effectively than Viosterol (A.R.P.I. process) per se. Klein and Russell ('31) have shown that the vitamin in irradiated ergosterol is somewhat more efficiently absorbed than that of cod liver oil in the chicken. The governing factor in the low potency of vitamin D₂ for the chick as found by means of the comparative technic appears to be one of utilization, since cod liver oil and vitamin D₃ are correspondingly potent in the rat and in the chicken. Thus it appears that the chemical differences between vitamin D₂ and D₃ are of primary importance in explaining the relative therapeutic effectiveness of these compounds in the chicken.

SUMMARY

1. Crystalline vitamin D₃ assayed by the U.S.P. antirachitic vitamin D test on rats has a potency of 0.025 γ per unit or 40,000,000 units per gram. This is identical with the potency of crystalline vitamin D₂.

2. The minimal protective daily dose for chicks fed a rachitogenic diet was found to be 2.5 I.U. of cod liver oil, 2.5 I.U. of crystalline vitamin D₃, 2.7 I.U. of irradiated cholesterol, 120 I.U. of Viosterol (A.R.P.I. process) and 85 I.U. of crystalline vitamin D₂.

3. The antirachitic ratio of effectiveness of cod liver oil to Vipsterol (A.R.P.I. process) rat unit for rat unit in the chick is 1:48. The ratio for vitamin D₃ to vitamin D₂ is 1:34.

4. The reasons for the variations in potency of vitamin D preparations when tested on rats and chicks are discussed.

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THE EFFECT OF ENHANCED IODINE INTAKE ON GROWTH AND ON THE THYROID GLANDS OF NORMAL AND GOITROUS RATS¹

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In a recent paper from this laboratory (Remington, Remington and Welch, '37) we presented extensive data on the fresh weight, dry matter and iodine content of the thyroid glands of rats reared on a commercial dog food,³ which has been found to contain 265 micrograms of iodine per kilogram, and which is an excellent diet for growth and reproduction. Since histological examination of sections from many glands revealed a normal 'resting' state, and since these glands were smaller and contained a higher percentage of dry matter than any others we have encountered, and were smaller than any reported in the literature, we considered the values found as typical for normal rats receiving a fully adequate allowance of iodine. It would be of interest, however, to determine whether increasing the iodine content of this diet, either by the addition of iodide or iodine-rich foods, would produce animals with still smaller glands of higher dry matter and iodine content.

In the work of Levine, Remington and von Kolnitz ('33 a) on the production of goiter by iodine deficient diets, the young rats used were produced on Russell's modification of the Sherman breeding ration, various lots of which were found to

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³ Made by Purina Mills, St. Louis, Missouri.

contain from 47 to 72 γ per kilogram of iodine. Although not fully apparent at that time, it appears in the light of later work that this diet produced rats with definitely enlarged thyroids with relatively low dry matter and iodine content. Hence in the work cited, as well as that on the iodine requirement of the rat (Levine, Remington and von Kolnitz, '33 b), the young rats used were already moderately goitrous, and so the technic must now be considered as of a curative rather than a preventive nature. In that work, when diets containing 400 γ per kilogram of iodine were fed, thyroids were obtained weighing 12.6 to 13.1 mg. per 100 gm., containing 28.0 to 28.3% dry matter and 0.228 to 0.304% of iodine, dry basis. The values which we now consider normal for rats weighing between 50 and 150 gm. are 10 mg. per 100 gm. in weight; dry matter 33%; iodine 0.200%. In reviewing the accumulated data of the laboratory over a period of 6 years, we have observed that whenever rats are reared on an iodine deficient regime and afterward placed on diets high in iodine, the glands will tend to return toward normal, but that either they are still somewhat enlarged and low in dry matter, or the iodine content is unusually high. This gives rise to the thought that with hyperplasia induced by iodine deficiency, the ability of the gland to store iodine may be increased, and suggests the desirability of determining the effect of feeding larger amounts of iodine to goitrous as well as normal rats.

EXPERIMENTAL

Following the same procedure employed in previous experiments, the young rats were placed on the various special diets at 3 to 4 weeks of age, and maintained for 5 weeks, after which they were killed by chloroform, the thyroids removed, weighed, and analyzed. Food intake and growth records were kept on all groups. Each group consisted of eight animals equally divided as to sex. In series I the iodine was added to the diet by evaporating an alcoholic solution of sodium iodide on a portion of the diet so as to yield a concentrate containing 35 γ of iodine per gram. This concentrate was then

mixed with the basal diet (ground dog biscuit) in proportions of 1, 2, 4, 8, 16 and 32%, for feeding to groups A to F respectively. The iodine content of the final mixed diets was calculated from the 265 γ per kilogram in the original, and the amount of concentrate added. In series II the iodine was added in the form of ground dried haddock, which contained 29.6 γ of iodine per gram, and which was added at 2.5 and 5% of the diet of groups G and H respectively. This was done because Remington, Coulson and Levine ('36) have found that at lower levels the iodine of haddock is fully utilized by the thyroid.

In series III the rats used were of the second generation of goitrous animals reared on our iodine deficient diet 342 (Remington, '37). A group sacrificed at the beginning of the feeding period yielded thyroids weighing 25.2 ± 1.8 mg. per 100 gm., and of dry matter 22.0%, iodine 0.024% respectively, and hence could be considered fairly representative of rats made goitrous by iodine lack. Groups B' and C' parallel groups B and C of series I. Group G' received the very large dosage of 25% of dried haddock. The results are presented in tabular form.

INTERPRETATION

In series I and II, in which the young rats were reared on the colony ration and hence presumably had normal thyroids from birth, it will be noted that there is no consistent change in any of the constants on the thyroid gland as the iodine content of the diet is increased up to forty times the 3 γ per day furnished by the colony ration. These rats, at the conclusion of the experiment, averaged about 180 gm. in weight, and from the work cited in the first paragraph, the normal values on colony diet alone would be: fresh weight 8.8 ± 0.40 per 100 gm., dry matter 33.3%, iodine 0.200%. The glands of the present series are smaller, and the iodine content higher. However, we have seen some evidence that the thyroids of summer rats, even on the same diet, are smaller than those of winter rats, and in fact a group of ten animals

(group O) continued on the colony ration simultaneously with this experiment in July and August 1937, gave, for thyroid weight 7.4 ± 0.29 , for iodine 0.183. It cannot be concluded that the enhanced iodine intake has brought about any changes in the thyroid, with the exception of a possible slight increase

TABLE 1
Effect of increased iodine intake on the thyroid glands of normal and goitrous rats

GROUP	IODINE IN DIET	DAILY FOOD INTAKE	IODINE INTAKE PER DAY	DAILY GAIN IN WEIGHT	FOOD FOR EACH GRAM OF GAIN	THYROID GLANDS		
						Fresh weight milligrams per 100 gm. body weight	Dry matter	Iodine dry basis
Series I. Iodine added as sodium iodide								
O ¹	γ per kg. 265	gm. 11.5	γ 3.0	gm. 3.77	gm. 3.05	7.4 ± 0.29	% 34.1	% 0.183
A	612	11.3	6.9	3.57	3.16	7.4 ± 0.25	32.9	0.233
B	960	11.3	10.8	3.63	3.12	7.5 ± 0.24	31.8	0.213
C	1304	11.1	14.5	3.63	3.05	7.3 ± 0.18	29.1	0.199
D	2344	11.7	27.4	3.69	3.16	8.0 ± 0.38	33.8	0.223
E	4423	12.0	53.1	3.71	3.20	8.1 ± 0.42	34.1	0.195
F	11380	10.7	121.7	3.63	2.96	7.2 ± 0.31	35.0	0.262
Series II. Iodine added as dried haddock containing 26.6 γ per gram								
G	997	12.4	12.4	3.89	3.20	7.9 ± 0.28	33.2	0.185
H	1730	11.3	19.5	3.91	2.90	6.8 ± 0.20	34.0	0.250
Series III. On second generation of goitrous rats								
N.C. ²						25.2 ± 1.80	22.0	0.024
B'	960	10.0	9.6	3.43	2.91	8.0 ± 0.32	30.0	0.322
C'	1304	10.0	13.0	3.34	3.00	8.9 ± 0.39	29.9	0.288
G'	7600	10.3	78.3	3.69	2.79	8.3 ± 0.22	29.0	0.281

¹ Basal diet without added iodine.

² Parallel group at beginning of experiment.

in the iodine content. It is also worthy of note that growth and the utilization of food for growth are not affected by amounts of iodine as high as 50 to 100 times the minimal protective dose of 1 to 2 γ per day, which amount, however, is still far below that which would bring about any pharmacodynamic effect.

Series III, in which the iodine enriched diets were fed to rats previously suffering from iodine deficiency, also shows nearly complete recovery as to weight and dry matter content of the thyroids, but unusually high iodine values. We have found in other experiments that diets which contain enough iodine to prevent thyroid enlargement, will not restore normal values in rats already goitrous. In one such experiment iodide was added to diet 342 (iodine deficient) so as to yield 5 γ per each 10 gm. of diet, or 5 to 6 γ per rat per day, and this diet fed for 6 and 14 weeks, respectively, to severely goitrous young rats. Parallel groups were maintained on diet 342 without the added iodine.

TABLE 2
Effects of iodide feeding on the thyroid glands of goitrous rats

	SERIES A CURATIVE PERIOD 6 WEEKS				SERIES B CURATIVE PERIOD 14 WEEKS			
	Number of animals	Thyroid weight per 100 gm.	Dry matter	Iodine dry basis	Number of animals	Thyroid weight per 100 gm.	Dry matter	Iodine dry basis
Before experiment	8	27.0 \pm 1.08	%	%			%	%
Without iodide	8	25.9 \pm 1.57	22.2	0.007	10	31.0 \pm 1.25	22.2	0.007
With iodide	10	11.9 \pm 0.42	28.8	0.195	8	12.1 \pm 0.41	28.4	0.164
Normal values		8-10	33	0.200				

Here we did not get abnormally high iodine values in the glands, but neither did we get full recovery as evidenced by weight and by dry matter content. A comparison of the data of the two tables reveals that when relatively large amounts of iodine are fed to goitrous rats, the glands return to normal or near normal in size and dry matter, but contain an abnormally high percentage of iodine; while if the amount given is smaller, yet still well above the preventive requirement, the return to normal is not complete so far as weight and dry matter content are concerned, but may be so as to iodine.

Other cases of abnormally high iodine content in glands which were slightly larger and more edematous than our normals have been encountered in this laboratory, but always

in rats that had a history of more or less severe iodine depletion previous to the iodine feeding period. Reference has already been made to the work of Levine, Remington and von Kolnitz in which the lowest thyroid weight obtained was 11.8 mg. per 100 gm., the highest dry matter 28.3%, but the highest iodine 0.304% in one group of ten rats. One of us spent of the summer of 1937 at the University of Minnesota, where we observed that ten rats from the colony of Dr. C. M. Jackson, killed at 9 weeks of age, yielded thyroids weighing 12.2 ± 0.24 mg., and of 27.4% dry matter and 0.102% iodine content. According to our criteria these must be considered as moderately goitrous and iodine deficient. A parallel group of these animals, transferred to a diet richer in iodine at 4 weeks and maintained thereon for 5 weeks longer, gave values of 9.1 ± 0.42 , 31.0 and 0.327 respectively. The average weight of these animals was 157 gm., hence the glands are of normal size, but the iodine value is 160% of normal.

SUMMARY AND CONCLUSIONS

The presence of 265 micrograms of iodine per kilogram in an otherwise well-balanced diet is sufficient to protect growing rats from iodine deficiency as evidenced by changes in the thyroid gland.

Further enhancement of the iodine content of the diet either by sodium iodide or dried haddock, up to forty times the amount present in the original, does not significantly affect the weight or dry matter content of the glands, but may possibly bring about a slight increase in the iodine content.

Iodine in the diet, up to 50 to 100 times the minimal protective dose, does not affect growth or utilization of food for growth.

If iodine enriched diets are fed to rats with enlarged and hyperplastic thyroids, the gland tends to return to normal weight and dry matter content, but such glands can store more iodine than normal ones.

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THE ROLE OF VITAMIN D IN THE CONTROL OF DENTAL CARIES IN CHILDREN

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ONE FIGURE

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Data on nutritional control of structure and stability of teeth are steadily accumulating. The literature has been reviewed at various times (Mellanby, '34; Com. for Invest. of Dental Disease, '36; Boyd, Drain and Nelson, '29). The present paper reports observations, principally on the effects of vitamin D, covering 4 successive years (1930 to 1934). Some of the material has been published (McBeath, '32, '34). It is now possible to correlate all the results and give a clearer picture both of caries incidence as encountered under our conditions and of the effects of vitamin D.

The original plan at the beginning of the 4-year study was to collect data during a school year. On this basis we had relative assurance that the groups would remain intact. During the first year three schools were available, each furnishing one experimental and one control group. The aim was to have twenty to thirty children in each group. As the work progressed it was found that quite a number of children was available during several successive years. This automatically extended the control observation periods over the summer, allowed us to study some of the children both as control and experimental subjects and opened up the question of seasonal effects.

The schools selected were orphanages in and near New York City, permanent homes of the children studied. Two are in the Borough of Brooklyn (St. John's Home and St. Joseph's Female Orphan Asylum); four are just outside the city limits

(Graham School, Hastings-on-Hudson, St. Christopher's School and the Children's Village in Dobbs Ferry, and Leake and Watts Home School in Yonkers, N. Y.). Three schools are conducted on the cottage plan (St. Christopher's School, Graham School, Leake and Watts Home School). The ages of the children ranged from 6 to 14 years.

THE MEASURE OF CARIES INCIDENCE

Various plans for recording carious activity in the teeth have been adopted. Several British investigators reported (Mellanby, '34; Com. for Invest. of Dental Disease, '36) the per cent of carious teeth as a fraction of the total number of teeth non-carious at the first examination and also estimated the intensity of the process by means of arbitrary numerical designations which were summated for the final result. In the present study a plan was adopted which in a sense combines these two features in one score.

Each mouth was subjected to a thorough examination including the teeth and the gums. With regard to the teeth a record was made of quality and color of the enamel, stains, cleanliness and caries, or abnormal conditions. Caries were recorded according to the involvement of the five surfaces: 1) mesial, 2) distal, 3) buccal or labial, 4) lingual, and 5) occlusal or incisal, as first suggested by Bodecker. The present report deals exclusively with the progress of caries as indicated by the increase in the number of surfaces involved in carious lesions. Any fissure deep enough to catch the explorer was recorded as carious. The record, therefore, included every detectable definite lesion of the enamel. Lesions involving more than one surface were outlined on charts according to their location and were scored according to the number of surfaces involved. Fillings were also scored by the number of surfaces involved. The error due to extension by operative intervention is apparently small.

The progress between two examinations is reported as the number of new carious surfaces per 100 days per mouth. In each individual child the number of carious surfaces found was noted on a suitable dental chart, and after the completion

of the year's observations the charts showed the number of new carious surfaces which appeared during each interval. Next on the basis of the number of days between examinations the number of new carious surfaces in each individual was calculated per 100 days. The score for the group was then determined as the mean number of new carious surfaces per 100 days per mouth.

The general plan was to conduct the first examination in the autumn, the second during the later part of the winter and the third final examination late in spring. In the studies of 1931-1932 the mid-winter examination was omitted. The dates of examinations are given in table 1.

Periods between examinations are designated as:

Autumn-winter period, the period between the first and second examinations, usually 100 to 180 days.

Winter-spring period, the period between the second and third examinations, usually 60 to 90 days.

Autumn-spring period, the period between the first and third examinations, usually 150 to 250 days (autumn-winter and winter-spring periods together).

Summer period, the period between the third (spring) examination of one school year and the first (autumn) examination of the following school year.

One year period, the period between an autumn examination of 1 calendar year and the autumn examination of the next calendar year.

Since in the groups here reported the average age and the average number of teeth per mouth are relatively constant and since the groups are of fair size, the results for a group can probably be safely expressed on a mean rate basis in terms of new carious surfaces per 100 days per mouth. This is called the 'score.' Separate classification by age, weight, rate of growth, previous condition of caries and average distribution of deciduous and permanent teeth showed no trends that disturbed the significance of the mean values of the 'score.' A number of interesting findings, similar in part to those of Anderson et al. ('34), are brought out by these tabulations and will be reported separately.

TABLE 1

Data chronologically arranged

SCHOOL		DATES OF EXAMINATION		MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH (871 CASES—270 CONTROLS, 601 EXPERIMENTALS)			
				Autumn-spring		Autumn-winter	
		Experimental	Control	Experimental	Control	Experimental	Control
1930-1931	Diet + vit. D ¹	11/7 -3/3 -5/20	11/7 -3/3 -5/20	0.74	2.12	0.71	2.39
	Diet + vit. D ¹	11/21-3/13-5/28	11/21-3/13-5/28	0.39	1.88	0.53	1.22
	Diet + vit. D ¹	11/14-3/17-6/8	11/14-3/17-6/8	0.49	1.80	0.43	0.93
1931-1932	Diet + vit. D ¹	12/11-5/20	12/11-5/20	0.32	1.28		
	Diet + vit. D ¹	12/8 -5/17	12/4 -5/17	0.33	1.49		
	Diet + vit. D ¹	11/27-5/28	12/7 -5/24	0.26	1.65		
(X)	Vioosterol ²	12/3 -5/27	12/3 -5/27	1.56	1.93		
	U. V. light on skin ⁴	12/3 -5/27		0.27			
1932-1933	Diet ⁵	12/6 -3/3 -5/12	12/6 -3/10-5/16	1.55	2.45	1.21	2.38
	Vioosterol ²	11/11-2/28-5/5	11/3 -3/7 -5/9	1.52	2.92	1.31	1.88
	U. V. light on skin ⁴	11/18-3/7 -5/2		0.97		0.88	
1933-1934	Milk ⁶	9/29-2/8 -4/17	9/25-2/8 -4/17	1.03	2.32	0.97	1.71
	Milk + 400 U. ⁸	9/25-2/6 -4/17		0.76		0.75	0.78
	Fresh vit. D milk ¹⁰	9/29-2/7 -4/17		1.16		0.98	1.51

(C)	Milk *	9/26-2/26-4/19	19	2.86	2.17	4.86
(C)	Milk + 400 U. ^a	9/26-2/26-4/18	22	0.98	0.69	1.86
(C)	Fresh vit. D milk ²⁰	9/26-2/26-4/18	24	0.92	0.65	1.72
(Y)	Milk *	10/9 -1/31-4/26	30	1.61	0.85	2.64
(Y)	Milk + 250 U. ⁷	10/9 -1/31-4/26	30	1.59	0.99	2.39
(Y)	Milk + 800 U. ⁸	10/9 -1/31-4/26	28	0.39	0.22	0.63
(Z)	Milk *	10/16-2/21-4/23	28	2.17	1.53	3.51
(Z)	Milk + 250 U. ⁷	10/18-2/23-4/24	24	1.09	1.72	2.57
(Z)	Milk + 800 U. ⁸	10/18-2/23-4/24	30	0.64	0.70	0.50
(Z)	U. V. light on skin ⁴	10/11-2/31-4/24	26	0.96	0.95	0.99
			601			
			270			

School (A), Graham; school (B), St. Christopher's; school (C), Leake and Watts; school (X), Children's village; school (Y), St. Joseph's; school (Z), St. John's.

¹ Revised diet (according to Boyd and Drain) with 3 teaspoonfuls of cod liver oil (probably 800 to 1000 international units) per day.
² Viosterol expressed at the time in Steenbock units (approximately 3000 international units per day). Institutional diet.
³ Viosterol expressed at the time in Steenbock units (approximately 6000 international units per day). Institutional diet.
⁴ Exposure twice weekly of both back and chest to a quartz mercury lamp. Institutional diet.
⁵ Revised diet (according to Boyd and Drain) with no cod liver oil.

⁶ One pint of plain reconstituted evaporated milk.
⁷ One pint of plain reconstituted evaporated milk containing 250 international units of natural vitamin D.
⁸ One pint of reconstituted evaporated milk containing 400 international units of natural vitamin D.
⁹ One pint of reconstituted evaporated milk containing 800 international units of natural vitamin D.
¹⁰ One pint of reconstituted evaporated milk containing 1600 international units of natural vitamin D.
¹¹ One pint of milk per day in which 400 international units of natural vitamin D had been incorporated at the dairy plant.

DATA CHRONOLOGICALLY ARRANGED (table 1)

1930-1931. In three schools a comparison was made between the effects of the ordinary uncorrected school diet and a diet modelled after that of Boyd, Drain and Nelson ('29) with the difference, however, that 3 teaspoonfuls of cod liver oil were given instead of one. The school diet and the revised diet have been discussed previously (McBeath, '32). Scrupulous supervision with regard to actual intake particularly of the protective foods was carried out by those in charge at table. It will suffice here to say that the school diet was the usual type commonly found in boarding schools with no obvious defects but being short of the optimum allowance of milk, fresh fruits and vegetables. The children were well nourished and healthy according to the current standards. The revised diet supplied an ample allowance of milk (1 quart per day), fresh fruits and vegetables together with a high level of vitamins A and D in the form of cod liver oil (about 800 U.S.P. units of D).

The caries incidence was obviously less in groups receiving the revised diet, thus confirming the findings of Boyd, Drain and Nelson ('29). A direct numerical comparison with these authors is not possible. Boyd and Drain reported principally on the occurrence and arrest of dentinal caries. Our data include enamel caries even in the incipient stages.

1931-1932. During this year the observations of 1930 to 1931 were repeated in the same three schools. As far as possible the groups were reversed so that those which had been 'controls' during the previous year became 'experimental' and vice versa. The data on such reversal of groups are given in table 4.

Only two examinations were made. The results are very similar to those of the previous year.

Two new types of experiments were added. One involved the administration of viosterol and the other the exposure of the skin of chest and back twice weekly to a quartz mercury lamp. The viosterol gave a moderate reduction of caries incidence. The ultraviolet light exposure had an effect com-

parable to that of the feeding of cod liver oil. For details see tables 6 and 7.

1932-1933. During this school year studies with ultra-violet light and viosterol were repeated with results similar to those of the previous year. The viosterol effect again did not equal that of cod liver oil, in spite of the fact that the viosterol dose was doubled.

At school (A) a new type of experiment was introduced, namely the feeding of the Boyd and Drain diet without addition of cod liver oil. This resulted in a distinct but moderate reduction in caries incidence which, by no means, reached the low values recorded when cod liver oil was included.

During this winter the first attempts were made to work with vitamin D concentrates from natural sources. Several lots of concentrates had to be used which turned out to be not of equal potency, and not being used simultaneously in the several groups, the effect, although evident, was very irregular. For this reason the data are omitted from table 1. The following year better control was achieved and larger groups of children were available.

1933-1934. As can be seen from table 1 the work was planned so as to include enough children to yield (combined) groups of about fifty on each phase of the experiment. A batch of carefully assayed vitamin D concentrate, enough to serve for the entire experiment at three levels of vitamin D intake, was incorporated in evaporated milk. These three vitamin D milks and also plain evaporated milk were put up in $\frac{1}{2}$ gallon tins. At each school each day the milk to be used was diluted with equal quantities of water and served to the children in the respective groups in the middle of the morning and in the afternoon. To make the milk consumption least burdensome for those children who were not fond of milk, and to secure the best results in regularity of intake, the milk was slightly flavored with chocolate. Each milk was repeatedly assayed and found to be of the stated potency.

The two 8-ounce glasses consumed contained the daily vitamin D intake given in the tables. In the preliminary paper

(McBeath, '34) this was given in terms of Steenbock units. At the time of introduction of the international standard a conversion factor of 2.7 was suggested on the basis of the average rat reaction in a number of laboratories. A re-check of the milk by means of the international standard showed that our rats at the time were of average healing susceptibility to vitamin D, and that the milk was of the potency stated in table 1.

Table 1 shows that the feeding of the plain evaporated milk gave a slight reduction in caries incidence and that increasing vitamin D intake with the milk gave progressively further reduction. This is shown in more detail in table 5.

Two other groups of children received 1 pint of fresh milk per day in which 400 U.S.P. units of vitamin D had been incorporated in the dairy plant.

TABLE 2

Summary of controls. Experiments in which three examinations were made

	INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH							
	1930-1931		1932-1933		1933-1934		All years	
	Number of cases	Score	Number of cases	Score	Number of cases	Score	Number of cases	Score
<i>Autumn-spring</i>								
(A) Graham	18	2.12	21	2.45	19	2.32	58	2.31
(B) St.Christopher's	11	1.88					11	1.88
(C) Leake and Watts	27	1.80	20	1.66	17	2.64	64	1.98
(Y) St. Joseph's					30	2.73	30	2.73
(Z) St. John's			26	2.92	29	3.09	55	3.04
Totals	56	1.92	67	2.40	95	2.74	218	2.43
<i>Autumn-winter</i>								
(A) Graham	18	2.39	21	2.38	19	1.71	58	2.17
(B) St.Christopher's	11	1.22					11	1.22
(C) Leake and Watts	27	0.93	20	0.77	17	2.35	64	1.26
(Y) St. Joseph's					30	2.92	30	2.92
(Z) St. John's			26	2.79	29	2.45	55	2.61
Totals	56	1.46	67	2.06	95	2.43	218	2.07
<i>Winter-spring</i>								
(A) Graham	18	1.71	21	2.56	19	3.56	58	2.62
(B) St.Christopher's	11	2.87					11	2.87
(C) Leake and Watts	27	3.08	20	2.94	17	3.51	64	3.15
(Y) St. Joseph's					30	2.47	30	2.47
(Z) St. John's			26	3.17	29	5.54	55	3.90
Totals	56	2.60	67	2.91	95	3.51	218	3.09

SUMMARY OF CONTROLS (table 2)

Autumn-winter, winter-spring, autumn-spring periods

The first section of the table compares the first (autumn) examination with the last (spring) examination for each school in each year in which three examinations were made. The two other sections of the table deal with the same observations when these are divided into two sub-periods (autumn-winter and winter-spring). The grand mean score for all schools and all years for the autumn-spring period was 2.43. Considering the fact that neither the diet nor the sunlight exposure was necessarily identical from year to year and school to school variations in the group scores are not surprising.

The results for the autumn-winter and winter-spring periods demonstrated the interesting fact that the caries incidence during the winter-spring period was distinctly higher. The grand total for the autumn-winter period was 2.07, the grand total for the winter-spring period was 3.09. Individual group scores may fall out of line but whenever mean values derived from fifty or more individuals are compared the winter-spring caries incidence is seen to be distinctly higher than that for the autumn-winter period.

The unequal length of periods might be considered open to criticism. It came about largely through the wish to accommodate ourselves to various routine arrangements in the schools. If a period were chosen too short to allow measurable signs of new caries to appear, the score per 100 days would be low. When, therefore, the shorter of the two periods shows the higher score, this cannot be attributed to the shortness of the period.

SUMMER CONTROLS AND CONTROLS UNDER OBSERVATION FOR A YEAR (table 3)

Our understanding of the seasonal effect is considerably extended when we observe those cases which were 'control' during an entire calendar year. Eighty-five individuals have been 'controls' during one school year and were again available the next fall. Seventeen of these came from the 1931-

TABLE 3
Summer controls and controls under observation for a year

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH				
	Summer	Whole year	Autumn-spring	Autumn-winter	Winter-spring
182 summer controls (including those which were 'experimental' during the previous year)	0.64				
85 controls under observation for a year (omitting the mid-winter examination)	0.57	1.41	2.22		
68 controls under observation for a year (having mid-winter examination) (included in the above)	0.64	1.51	2.32	1.74	3.12

1932 experiments where only two examinations were made. This reduced the recorded autumn-winter and winter-spring entries to sixty-eight. One hundred eighty-two cases, including the 'experimentals' of the previous year, were summer controls with a score of 0.64. This contrasts sharply with the value of 3.12 for the winter-spring period.

TABLE 4
Comparison of experimental and control regime in the same individual

GROUP	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH AUTUMN-SPRING PERIOD		
	Number of cases	Experimental	Control
1930-1931 improved diet + 3 teaspoonfuls cod liver oil	33	0.62	1.46
1931-1932 improved diet + 3 teaspoonfuls cod liver oil	33	0.26	2.25
1933-1934 400 U. vitamin D milk	39	0.63	1.64
All cases	105	0.52	1.75

COMPARISON OF EXPERIMENTAL AND CONTROL REGIME IN THE SAME INDIVIDUAL (table 4)

Table 4 comprises the 105 cases which appeared in the study as controls during 1 year and as experimental subjects during

another. When 'experimental' they were distributed between three groups with daily vitamin D intake as follows:

1930-1931, 3 teaspoonfuls of cod liver oil—33 individuals

1931-1932, 3 teaspoonfuls of cod liver oil—33 individuals

1933-1934, 400 international units of vitamin D in milk—39 individuals

The control observations were made either the year before or the year after the experimental observations.

It will be seen that the same individuals when in control groups in which no added source of vitamin D was given produced a score of 1.75 while with added vitamin D they scored 0.52. Many of the control values date from years and schools where caries incidence was generally low.

TABLE 5
Graded doses of vitamin D given as vitamin D milk

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH							
	Number of cases		Autumn-spring		Autumn-winter		Winter-spring	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
250 U. vitamin D milk (evaporated)	54	59	1.77	2.91	1.32	2.69	2.47	3.49
400 U. vitamin D milk (evaporated and fluid)	89	36	1.20	2.49	0.77	1.99	1.47	3.64
800 U. vitamin D milk (evaporated)	58	59	0.52	2.91	0.47	2.69	0.56	3.49
Plain evaporated milk	95	95	1.91	2.74	1.34	2.42	3.06	3.55

GRADED DOSES OF VITAMIN D GIVEN AS VITAMIN D MILK (table 5)

These experiments were performed in the winter of 1933-1934 with three different levels of vitamin D intake representing 250, 400 and 800 international units per day. In the case of each group the results were compared with a similarly constituted group which was left on the institutional diet in the same school at the same time. The experiment was so conducted that the several levels of vitamin D intake were distributed in groups of twenty to thirty individuals between four different schools. This resulted in the same individuals serving as controls for the 250 unit and 800 unit levels (see

table 1). Each vitamin D level was represented by at least fifty individuals.

It is clearly seen that there is a gradation of caries incidence with the amount of vitamin D added to the diet. A comparison of the autumn-winter and winter-spring period of study is of particular interest. When we consider the control data it is seen that in each case the winter-spring period gave higher values than the autumn-winter period. In the groups receiving 250 units of vitamin D the autumn-winter period resulted in a score of 1.32 against 2.47 for the winter-spring period, and the mean value for the whole autumn-spring period was reduced only moderately below the value for controls. In the 400 unit groups the reduction below the control value was considerable, but there was still a difference between the autumn-winter and the winter-spring period. This would indicate that 400 units per day is sufficient to keep the caries incidence to low values during the autumn-winter period, but not during the winter-spring period. With 800 units of vitamin D the difference between the autumn-winter and winter-spring period is negligible.

TABLE 6
Irradiated ergosterol (viosterol)

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH AUTUMN SPRING PERIOD			
	Number of cases		Score	
	Experimental	Control	Experimental	Control
(X) Children's Village (1931-1932)	21	23	1.56	1.93
(Z) St. John's School (1932-1933)	21	26	1.52	2.92
Totals	42	49	1.54	2.45

IRRADIATED ERGOSTEROL (VIOSTEROL) (table 6)

The data on the feeding of viosterol without any further change in the diet reveal a caries inhibiting effect, although not as marked as that produced by exposure of the skin to ultraviolet light with similar food regime.

As with cod liver oil, the possibility of the viosterol not being swallowed was guarded against from the beginning. The nurse required that 'thank you' be clearly enunciated

after the viosterol was received on the tongue and the children remained in the room for some time after.

This topic deserves more study since, as far as we know, a direct comparison of definitely known amounts of viosterol and vitamin D from cod liver oil has not been made.

TABLE 7
Exposure of the skin to ultraviolet light

	MEAN INCREASE IN CARIOUS SURFACES PER 100 DAYS PER MOUTH							
	Number of cases		Autumn-spring		Autumn-winter		Winter-spring	
	Experi- mental	Control	Experi- mental	Control	Experi- mental	Control	Experi- mental	Control
(X) Children's Village (1931- 1932) ¹	19	23	0.27	1.93				
(Z) St. John's School (1932- 1933)	27	26	0.97	2.92	0.88	2.79	1.13	3.17
(Z) St. John's School (1933- 1934)	26	29	0.96	3.09	0.95	2.45	0.99	4.54
Totals for three examinations	53	55	0.97	3.01	0.92	2.61	1.06	3.90
Totals for two examinations	72	78	0.78	2.69				

¹ In 1931-1932 only two examinations were made—autumn and spring.

EXPOSURE OF THE SKIN TO ULTRAVIOLET LIGHT (table 7)

To prove beyond question that additional vitamin D brings about certain stated results we should make a change in only this one factor. Natural vitamin D as a chemical individual was not available at the time these studies were carried out. Considerable extra weight can be attached to the evidence for vitamin D being a potent factor in caries prevention by the experiments on exposure of the skin to ultraviolet light.

For the ultraviolet light treatment a quartz mercury lamp was used. The distance from the lamp to the body was 60 inches. Two exposures per week were made on both chest and back. The first exposure was $\frac{1}{2}$ minute on chest and $\frac{1}{2}$ minute on back. Each week the exposure time was increased

by $\frac{1}{2}$ minute, leading to a final exposure of 12 minutes. None of the children experienced any discomfort at any time.

The caries-inhibiting effect of exposure to ultraviolet light as shown in the table is in good agreement with the implications of the summer control data. With some variation from year to year the mean score is 0.78 for seventy-two subjects when the whole autumn-spring period is recorded. The corresponding control value is 2.69 for seventy-eight individuals. Only two groups had the intermediate dental examination. For these the winter-spring period values are probably not significantly higher than those for the autumn-winter period.

DISCUSSION

The results here reported verify the findings of Mellanby and others regarding the effect of vitamin D on incidence of dental caries. On the quantitative side both the optimal dose and the type of vitamin D require further investigation. It would also seem very desirable if a method acceptable to all investigators could be devised and agreed on for the reporting of caries incidence. While the method here employed gives consistent results, there is no reason to believe that in its present form it is the best procedure.

There is no question that the chance for optimal tooth structure and tooth stability is the greatest when an 'ideal' diet is fed. The marked effect of vitamin D, however, together with observations on the effect of season and exposure to ultraviolet light lend much support to the thought that the calcium-phosphorus-vitamin D interrelationship plays a specific role in processes of tooth growth and preservation. This is in no way at variance with the observations that in some species (guinea pig) a vitamin C deficiency can produce marked carious lesions in teeth. Vitamin C and vitamin D are the two factors whose deficiency also produces specific lesions in bones.

When Mrs. Mellanby's first caries studies appeared, immediate ready acceptance was not generally accorded because other theories which excluded nutritional effects were very firmly entrenched. The significance of any measurement of

caries incidence or caries prevention was also an open question. To all corroborative evidence published since that time we can add our observations, which entirely confirm Mrs. Mellanby's judgment on both these points.

Considering all facts adduced so far it seems logical to consider the condition of teeth (re caries) as an indicator of good or faulty nutrition. What particular deficiencies are responsible may vary geographically or with other living conditions. It appears, however, that under most conditions that phase of metabolism which is under the control of vitamin D occupies a very prominent role. This, of course, includes considerations of adequacy of calcium and phosphorus intake.

The question whether, in children, other than nutritional etiologic factors are operative cannot be argued fairly until diets optimal in every respect are devised and these are rigidly maintained for a sufficient length of time and on a sufficient number of individuals to obtain valid results.

The seasonal effect on caries incidence seems to be well established. The marked spontaneous reduction of new lesions during the summer emphasized the extent to which sun exposure without further dietary control affects the picture. This may be held to bear out the analogy between caries and rickets. A recent paper by Mills ('37) in which he indicates a correlation between geographical latitude and caries incidence, also points to available sunshine as playing a definite role. While the experiments with high vitamin D intake and especially the summer controls suggest that vitamin D effects (whether by mouth or through sunlight action) play a prominent role, the rest of the diet, especially the calcium intake, cannot be neglected. It will be of interest to see whether a regime optimal in calcium and vitamin D (or summer sunlight) and also otherwise well balanced will reduce caries incidence to zero. So far no regime has been shown consistently to prevent the occurrence of new carious lesions in a reasonably large group.

It is worthwhile to mention briefly the results of subjecting the data to some of the elementary methods of statistical checking. Besides the mean (M) we have determined the

mean deviation (ϵ) and the mean deviation of the mean (ϵ_M) for all the groups or combined groups and have calculated the probability ratio $\frac{M_a - M_b}{\sqrt{\epsilon_{Ma}^2 + \epsilon_{Mb}^2}}$ for all the important comparisons which might be made to establish validity. (For definition of terms see Scott ('27)). We have convinced ourselves that none of the conclusions drawn from the data need to be questioned on statistical grounds as far as size of groups and calculated deviations about the means are concerned.

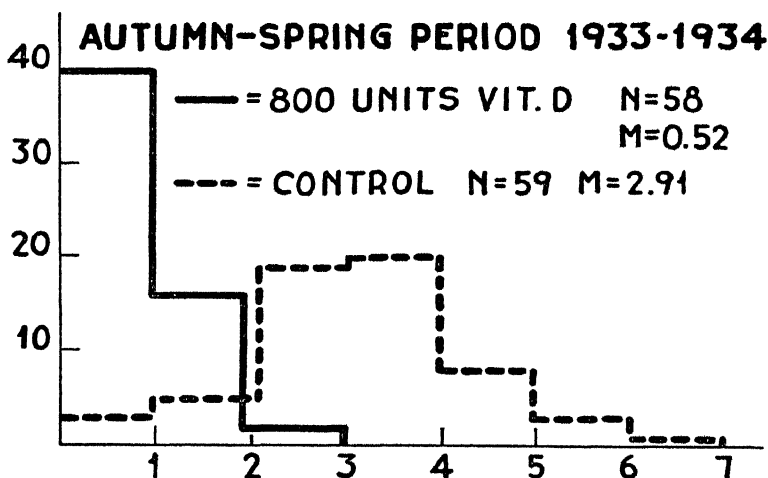


Fig. 1 Frequency polygons to illustrate the distribution of the various degrees of caries incidences in a control group of fifty-nine cases and a group receiving 800 units of vitamin D, fifty-eight cases. The number of new carious surfaces per 100 days is plotted against frequency.

For a comparison of the graded vitamin D milk data with the corresponding control values we find probability ratios ranging from 5 to 12 for all except the comparison of the 250 unit experiment during the winter-spring period, where the probability ratio is 2.2. This makes the last mentioned comparison just significant. The plain evaporated milk groups, which show mean values somewhat better than the controls, give probability ratios of 4 and 5 except during the winter-spring period where the value is below 2. This might be interpreted as meaning that during the height of the caries season the plain evaporated milk is not sufficiently protective

to differ from the controls while at times of less severe caries susceptibility its effect is slight but definite. The probability ratios for comparison of ultraviolet light treatment with its controls are very safe, all being above 6.

As far as the seasonal effect is concerned a comparison between the summer controls and any of the controls for the other periods gives probability ratios of 3 and 4. All values for the comparison of autumn-winter and winter-spring periods in control or experimental groups lead to probability ratios of 3 to 5 with the exception of the high vitamin D intake. In the case of the 800 unit experiments the ratio is 0.6, thus confirming the conclusion previously noted that only with a high vitamin D level of about 800 units will the difference between the autumn-winter and winter-spring periods be obliterated. This applies also to the comparison of the autumn-winter and winter-spring periods of 1930-1931 diet studies when the three schools are combined.

The type of change in the distribution of the number of new carious surfaces brought about in going from a control group to an 800 unit vitamin D group is illustrated in figure 1.

We wish to express our thanks to Dr. William A. Verlin for his very valuable assistance in the dental examinations. Grateful acknowledgment is also due to the Dean Milk Company of Chicago for the very kind gift of evaporated milk and vitamin D evaporated milk. Thanks are also due to the Ille Electric Corporation of New York for the loan and installation of the Burdick solarium lamp.

CONCLUSIONS

1. In a study comprising observations of over 800 children it was found that the incidence of dental caries was seasonal. The greatest incidence was found in late winter and early spring and very low values during the summer.
2. Previous observations on the beneficial effects of vitamin D were verified. The administration of graded amounts of natural (animal source) vitamin D as vitamin D milk

resulted in graded caries prevention. Of the three levels given (250, 400 and 800 international units per day), only the last named was adequate to prevent an increase during the height of the caries season above that of the previous period.

3. Fortifying the diet with 'protective foods' or simply increasing the allowance of milk in the diet led to a moderate reduction in caries when no appreciable vitamin D was added. The change is clear cut in the autumn-winter period but not so definite in the winter-spring period.

4. A reversal of 'control' and 'experimental' regimes during 2 successive years in over 100 cases showed that individual susceptibility to caries was negligible compared to the effect of nutritional factors.

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THE SPECIFIC DYNAMIC EFFECTS OF PROTEINS WHEN ADDED IN DIFFERENT AMOUNTS TO A MAINTENANCE RATION¹

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ONE FIGURE

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The influence of different levels of intake of protein on the heat production of an animal is of both physiological and nutritional interest. From the latter point of view this question is particularly important, inasmuch as the specific dynamic effect of a food nutrient affects its net energy value. Despite the numerous investigations of this subject on record, the available evidence concerning the dynamic effect of protein when fed in different quantities is not incontrovertible.

Rubner ('02) made the observation that the dynamic effect of a protein is proportional to the amount ingested. This was found to be essentially true by Williams, Riche and Lusk ('12), when feeding to a dog large quantities of meat.

On the contrary, in a series of experiments in which a man was fed casein exclusively, in varying quantities, Gigon ('11) found a progressive increase in the specific dynamic value per unit of the protein ingested, from the lowest to the highest level. Similarly, Weiss and Rapport ('24) observed that when increasing quantities of beef were given to a dog, the increase in heat production was not a linear function of the food ingested. This increase was found, however, to be, in general, directly proportional to the amount of protein metabolized.

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In all of the foregoing investigations the dynamic effect of protein was determined as the increase in heat production over the post-absorptive metabolism.

In the present study the problem was approached from a somewhat different angle, in accord with the following conception.

In a recent publication by Kriss, Forbes and Miller ('34) evidence was presented to show that the sparing action of food nutrients upon body nutrients, which takes place below maintenance, is likely to cause confusion of the apparent dynamic effects of these nutrients, and that, from the point of view of nutrition, more significant measurements of specific dynamic effects of food nutrients could be made if the heat production of an animal maintained in energy and nitrogen equilibrium, instead of the fasting metabolism, were used as the base value.

Consequently, in the experiments recorded herein, the main object was the determination of the specific dynamic effects of different proteins, as affected by the quantity fed, when these nutrients were given as supplements to a diet providing the requirements of energy and nitrogen equilibrium.

EXPERIMENTAL

The subjects of this investigation were twenty-four male rats, approaching maturity, and weighing approximately 200 gm. each. When this weight was reached the rats were removed from the stock colony and placed in individual cages. They were then fed 8.0 gm. per day of a basal ration composed of 93.7% of calf meal (described by Forbes, Kriss and Miller, '34) and 6.3% of butterfat. This diet maintained the rats at constant weight, and was found in a previous study (Kriss and Voris, '37) to be adequate to maintain rats of this size in approximate energy and nitrogen equilibrium.

The rats were divided into three groups of eight individuals, each group receiving a different protein food as the test substance. Group 1 received casein; group 2 received gelatin, while group 3 received heart muscle. Each of these substances was fed as a supplement to the basal ration in quanti-

ties of 1.5 gm. and 3.0 gm. All rats were subjected, at approximately weekly intervals, to respiration experiments while on each of the following daily dietary treatments, and in the order indicated: 1) fasting, 2) 8.0 gm. basal ration, 3) 8.0 gm. basal ration plus 1.5 gm. protein supplement, 4) 8.0 gm. basal ration, 5) 8.0 gm. basal ration plus 3.0 gm. protein supplement, 6) 8.0 gm. basal ration.

The particular substances used as the protein supplements, in the supermaintenance periods, were selected primarily because of the wide differences in their amino acid composition.

The chemical composition of the basal ration and of the protein supplements is given in table 1. The casein, labeled

TABLE 1
Composition of basal ration and protein supplements

MATERIAL	MOISTURE	NITROGEN	ETHER EXTRACT	ASH	ENERGY
	%	%	%	%	<i>calories per gram</i>
Basal ration	8.99	3.00	9.25	5.21	4358
Casein	9.15	12.99	0.22	0.92	5175
Basal ration	9.40	3.29	9.62	4.90	4362
Nutrient gelatin	8.17	15.22	0.13	3.48	4573
Basal ration	6.95	3.34	10.28	5.07	4447
Heart muscle	1.64	12.95	9.32	5.02	5559

'pure,' was obtained from Pfanstiehl. The gelatin was of the 'Barto Nutrient' grade, dehydrated. The heart muscle, as fed, was prepared from beef heart by removal of visible fat, freezing, drying in a Hempel desiccator, and grinding to a fine powder.

As stated above, the protein supplements were given in only two different quantities. The larger quantity (3.0 gm.) closely represented the maximum which all groups of rats would clean up, in addition to the basal ration, in the reasonably short time required by the method of experimentation. No smaller quantity than 1.5 gm. was tried, since significantly smaller quantities could not be expected to yield consistent heat increment values.

The proportions of protein ($N \times 6.25$) in the various rations, in relation to the total quantity of dry matter, were, therefore, as follows: for group 1—basal ration, 20.6%, basal ration plus 1.5 gm. casein, 31.4%, basal ration plus 3.0 gm. casein, 39.3%; for group 2—basal ration, 22.7%, basal ration plus 1.5 gm. gelatin, 35.6%, basal ration plus 3.0 gm. gelatin, 45.0%; for group 3—basal ration, 22.4%, basal ration plus 1.5 gm. heart muscle, 32.3%, basal ration plus 3.0 gm. heart muscle, 39.4% protein.

The metabolizability of the basal ration and of the protein supplements, at the two levels, was determined in a separate series of experiments, the results of which have been published elsewhere (Kriss and Voris, '37).

The practice of giving the daily allowance of food in two equal portions was again followed. This method results in an approximately uniform rate of metabolism, the respiration measurements, carried on during several hours of the day, therefore, being satisfactorily representative of the entire 24 hours.

All respiration measurements were made in periods of 6 to 7 hours.

The fasting experiments began 24 hours after food, following a period of feeding on the basal ration of not less than a week. The determination of the fasting metabolism served as a basis for correcting the heat production of the subjects in all experimental periods to a basis of uniform body weight. Immediately following the measurements of the fasting metabolism the rats were placed back on the basal ration.

The respiratory metabolism representing the various rations was determined at the end of approximately 1 week's feeding on the ration to be tested. These measurements began in each case soon after the morning meal was consumed.

The weekly intervals were chosen in consideration of the uniformity and consistent character of the metabolic results obtained under similar conditions by Kriss, Forbes and Miller ('34), and in the light of subsequent work by Kriss and Voris (unpublished results) which showed that if rats are kept on

a constant supermaintenance diet for relatively long periods of time (2 weeks or longer) the dynamic effect of the diet may be obscured by secondary changes in the metabolism, resulting from the changes in age and in body weight.

As a check on the possible effect of age and body weight on the metabolism during the weekly periods, each supermaintenance period was preceded and followed by a period of maintenance feeding.

The schedule of respiration experiments and the body weights are presented in table 2.

The determinations of the respiratory exchange were carried out by means of the apparatus which was previously described by Forbes, Kriss and Miller ('34), but which has since undergone certain modifications. These included 1) the substitution of a constant temperature air-bath for the constant temperature water-bath, 2) provision for the visibility of the rats by the use of reflecting mirrors, and 3) the installation of a device for recording the activity of the subjects while in the respiration chamber. The latter device, however, was not installed until after the experiments with the casein group had been completed.

The activity recorder (fig. 1) consists of a 'work adder'² which is placed above the chamber and connected to it by means of a metal rod, the chamber (glass jar) being suspended by springs. Any vertical movement of the chamber is registered on the graduated disc of the work adder, while complete revolutions of this disc are registered on an improvised scale.

Readings of activity were recorded at hourly intervals corresponding to the measurements of the CO₂ production. These records aided somewhat in the interpretation of the hourly CO₂ measurements, and in the elimination of certain data, obviously affected by activity, from the average results.

The computations of the heat production were made in accord with the general procedure followed previously by Kriss, Forbes and Miller ('34), while the protein metabolism

² This was purchased from the Harvard Apparatus Co., Boston, Massachusetts.

TABLE 2
Schedule of respiration experiments and body weights

RAT NO.	1 FASTING		2 BASAL RATION 8.0 GM. DAILY		BASAL RATION PLUS 1.5 GM. CASEIN		BASAL RATION 8.0 GM. DAILY		BASAL RATION PLUS 3.0 GM. CASEIN		BASAL RATION 8.0 GM. DAILY	
	Date	Body weight gm.	Date	Body weight gm.	Date	Body weight gm.	Date	Body weight gm.	Date	Body weight gm.	Date	Body weight gm.
11	1936 May 5	201	1936 May 12	209	1936 May 20	221	1936 May 26	213	1936 June 2	238	1936 June 9	224
12	May 5	207	May 12	217	May 20	233	May 26	225	June 2	247	June 9	235
13	May 6	205	May 13	214	May 21	224	May 27	224	June 3	247	June 10	236
14	May 6	193	May 13	204	May 21	211	May 27	211	June 3	240	June 10	230
15	May 7	198	May 14	201	May 22	214	May 28	213	June 4	233	June 11	225
16	May 7	203	May 14	210	May 22	215	May 28	215	June 4	235	June 11	228
17	May 8	213	May 15	219	May 23	228	May 29	225	June 5	242	June 12	233
18	May 8	203	May 15	210	May 23	220	May 29	220	June 5	238	June 12	233
BASAL RATION PLUS 1.5 GM. GELATIN												
21	Nov. 9	171	Nov. 16	167	Nov. 23	181	Dec. 1	176	Dec. 10	196	Dec. 17	187
22	Nov. 9	187	Nov. 16	186	Nov. 23	198	Dec. 1	189	Dec. 10	212	Dec. 17	205
23	Nov. 10	191	Nov. 17	193	Nov. 24	208	Dec. 2	198	Dec. 11	222	Dec. 18	216
24	Nov. 10	178	Nov. 17	183	Nov. 24	193	Dec. 2	187	Dec. 11	206	Dec. 18	202
25	Nov. 11	169	Nov. 18	174	Nov. 25	182	Dec. 3	183	Dec. 14	207	Dec. 21	206
26	Nov. 11	167	Nov. 18	174	Nov. 25	188	Dec. 3	185	Dec. 15	204	Dec. 21	204
27	Nov. 12	165	Nov. 19	172	Nov. 27	185	Dec. 4	181	Dec. 15	188	Dec. 22	188
28	Nov. 12	176	Nov. 19	171	Nov. 27	179	Dec. 4	172	Dec. 14	191	Dec. 22	183
BASAL RATION PLUS 1.5 GM. HEART MUSCLE												
31	1937 March 2	188	1937 March 8	197	1937 March 15	208	1937 Mar. 22	206	1937 Mar. 30	225	1937 Apr. 5	226
32	March 2	187	March 8	198	March 15	209	Mar. 22	204	Mar. 30	226	Apr. 5	224
33	March 3	186	March 9	183	March 16	198	Mar. 23	198	Mar. 31	221	Apr. 6	215
34	March 3	187	March 9	191	March 16	203	Mar. 23	207	Mar. 31	234	Apr. 6	226
35	March 4	166	March 10	169	March 18	186	Mar. 24	183	Apr. 1	207	Apr. 7	202
36	March 4	192	March 10	191	March 18	211	Mar. 24	207	Apr. 1	229	Apr. 7	223
37	March 5	186	March 11	178	March 19	203	Mar. 25	206	Apr. 2	225	Apr. 8	222
38	March 5	177	March 11	179	March 19	197	Mar. 25	197	Apr. 2	224	Apr. 8	218

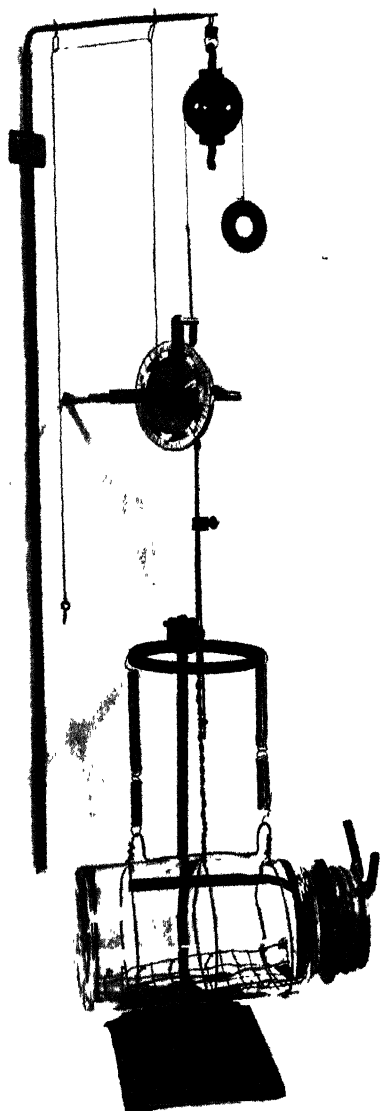


Fig. 1 Work adder connected with the respiration chamber.

was computed on the basis of the results obtained by Kriss and Voris ('37).

In order to provide a basis for calculating the heat production per unit of empty body weight, with each dietary treatment, the fill was determined in control animals which were of approximately the same age and weight as the experimental subjects.

RESULTS

The average hourly heat production of the individual rats, under the different dietary treatments, was computed, for comparative purposes, per 200 gm. of empty body weight. These data, as well as the heat increments resulting from the addition of the protein supplements to the basal ration, are set forth in table 3.

The heat production representing the basal ration exhibits a fair degree of uniformity among the different groups of animals, as well as among the animals of each group. The average results of each group show only a slight decline in metabolism from the initial to the final maintenance period, this decline being relatively greatest with the casein group. No significant difference is observed in the metabolism of the other two groups between the intermediate and final maintenance periods. Contributing to the uniformity of these results is the fact that no extensive changes in body weight occurred during the rather short experimental feeding periods.

No less consistent than the foregoing results are the data for heat production in the supermaintenance periods. In each case the addition of the protein supplement to the basal ration resulted in an increased metabolism, and, with only one exception (rat 21), the larger quantity of protein caused the greater heat increment.

The hourly increases in heat production of the individual rats, due to the supplements, are presented in the last two columns. In the computation of these heat increments the average heat production for two maintenance periods (one immediately preceding and one immediately following the supermaintenance period) was used as the base value. This

TABLE 3

Hourly heat production of rats per 200 gm. of empty body weight as influenced by addition of protein supplements to a basal maintenance ration

RAT NO.	BASAL RATION 8.0 GM. DAILY			BASAL RATION PLUS PROTEIN SUPPLEMENT		INCREASE IN HEAT PRODUCTION DUE TO SUPPLEMENT	
	Initial period	Inter-mediate period	Final period	1.5 gm. casein	3.0 gm. casein	1.5 gm. casein	3.0 gm. casein
	<i>calories</i>	<i>calories</i>	<i>calories</i>	<i>calories</i>	<i>calories</i>	<i>calories</i>	<i>calories</i>
11	840	830	792	975	1134	140	323
12	800	814	799	933	1078	126	271
13	891	820	789	934	1156	78	351
14	853	808	815	943	1112	112	300
15	877	842	809	989	1093	129	267
16	775	781	774	907	990	129	212
17	812	800	733	928	1007	122	240
18	851	826	788	967	1063	128	256
Average	837	815	787	947	1079	121	278
Standard deviation	36.8	17.8	23.8	25.7	54.3	17.7	42.3
	± 6.21	± 3.00	± 4.01	± 4.33	± 9.16	± 2.98	± 7.13
				1.5 gm. gelatin	3.0 gm. gelatin	1.5 gm. gelatin	3.0 gm. gelatin
21	838	903	839	975	973	104	102
22	846	879	821	936	1020	73	170
23	757	748	801	868	955	115	180
24	808	777	831	858	946	65	142
25	872	858	846	1006	1045	141	193
26	856	838	830	947	988	100	154
27	868	786	840	915	947	88	134
28	928	833	840	956	1002	75	165
Average	847	828	831	933	985	95	155
Standard deviation	46.7	50.0	13.5	47.4	33.8	23.6	26.9
	± 7.87	± 8.43	± 2.28	± 7.99	± 5.70	± 3.98	± 4.53
				1.5 gm. heart muscle	3.0 gm. heart muscle	1.5 gm. heart muscle	3.0 gm. heart muscle
31	899	917	884	981	997	73	96
32	839	836	830	895	961	57	128
33	839	818	807	902	976	73	163
34	841	852	801	903	992	56	165
35	965	849	860	996	1011	89	156
36	871	880	850	994	1021	118	156
37	904	819	869	945	1010	83	166
38	853	837	896	947	1035	102	168
Average	876	851	850	945	1000	81	150
Standard deviation	41.5	31.1	32.4	39.4	22.5	20.0	23.6
	± 7.00	± 5.24	± 5.46	± 6.64	± 3.79	± 3.37	± 3.98

procedure is believed to compensate for any slight changes in the rate of metabolism incidental to the changes in age and body weight.

It will be observed that the heat increments of the larger quantities of protein supplements are more consistent than those obtained for the smaller quantities. This is to be expected. In general, heat increments are subject to relatively large experimental errors, because their determination necessarily involves a comparison of results obtained in different experimental periods.

The increases in heat production caused by 3.0 gm. of casein are, with one exception (rat 13), approximately twice as great as those caused by 1.5 gm. casein. The average values for the entire group of eight animals are 121 calories per hour for the smaller quantity of casein, and 278 calories for the larger quantity of casein. If the aberrant results of rat 13 are omitted, the averages are 127 calories and 267 calories, respectively, these values being closely proportional to the quantities of protein supplement.

Gelatin in the amount of 1.5 gm. caused an average heat increment of 95 calories per hour as compared with the increase of 155 calories caused by 3.0 gm. of gelatin. The results with rat 21 present the only case in which the heat increment of 3.0 gm. of protein supplement was found not to be larger than the increment for 1.5 gm. protein supplement.

The ingestion of 1.5 gm. of heart muscle resulted in an average increase in heat production of 81 calories per hour, while 3.0 gm. of the same substance caused an increase of 150 calories per hour. These results also show an approximate proportionality between the quantity of food ingested and the dynamic effect.

In table 4 are presented the average results of the calculations of the specific dynamic effects and of the net energy values of the protein supplements at the two levels of feeding.

The values for metabolizable energy of the supplements represent the following percentages of the gross energy: for 1.5 gm. casein, 81.4%, for 3.0 gm. casein, 82.9%; for 1.5 gm.

TABLE 4
Specific dynamic effects and net energy values of casein, gelatin and heart muscle at two different levels of feeding

DAILY SUPPLEMENT	GROSS ENERGY	METABOLIZABLE ENERGY	TOTAL NITROGEN	NITROGEN RETAINED IN THE BODY	SPECIFIC DYNAMIC EFFECTS				NET ENERGY PER GRAM	
					PROTEIN KATABOLIZED		Per 100 cal. of protein katabolized	Per cent of metabolizable energy		
					Urinary nitrogen	mg.			calories	calories
							Per 24 hours	calories		
1.5 gm. casein	7,763	6,319	195	6.0	176	2904	52.8	46.0	2277	2181 ¹
3.0 gm. casein	15,525	12,870	390	17.2	310	6672	69.4	51.8	2066	2154 ¹
1.5 gm. gelatin	6,860	5,598	228	13.9	188	2280	50.4	40.7	2212	2308 ²
3.0 gm. gelatin	13,719	11,044	457	21.5	347	7925	47.8	34.3	2417	2377 ²
1.5 gm. heart muscle	8,339	7,372	194	29.1	130	3578	54.3	26.4	3619	
3.0 gm. heart muscle	16,677	14,159	389	28.6	260	6734	53.5	25.4	3523	

¹ Omitting results of rat 13.

² Omitting results of rat 25.

³ Omitting results of rat 21.

gelatin, 81.6%, for 3.0 gm. gelatin, 80.5%; for 1.5 gm. heart muscle, 88.4%, for 3.0 gm. heart muscle, 84.9%. These percentages are as determined by Kriss and Voris ('37).

The data representing protein katabolism were derived on the following basis: of the nitrogen ingested in the form of the supplements, the following percentages were found by Kriss and Voris ('37) to be excreted in the urine: for 1.5 gm. casein, 90.5%, for 3.0 gm. casein, 79.6%; for 1.5 gm. gelatin, 82.6%, for 3.0 gm. gelatin, 75.9%; for 1.5 gm. heart muscle, 67.0%, for 3.0 gm. heart muscle, 66.9%. The calorific values per milligram of urinary nitrogen were as follows: for 1.5 gm. casein, 31.27 calories, for 3.0 gm. casein, 31.03 calories; for 1.5 gm. gelatin, 24.04 calories, for 3.0 gm. gelatin, 22.84 calories; for 1.5 gm. heart muscle, 27.52 calories, for 3.0 gm. heart muscle, 25.90 calories.

The dynamic effects of the protein supplements are expressed 1) in calories per 24 hours, 2) in calories per 100 calories of extra protein katabolized, and 3) as per cent of the metabolizable energy of the supplements.

On all three bases the average results for all the animals used in these experiments are given. In some cases, as indicated, the average results for seven animals (omitting certain extreme results) are also included. This was done in order to bring out more clearly the significance of the data.

The results show that whether the specific dynamic effects of the proteins are expressed as per cent of extra protein katabolized, or as per cent of the total metabolizable energy of the protein supplements, no significant differences in the specific dynamic values resulted from the feeding of different quantities of the proteins.

The specific dynamic effect of casein, expressed as per cent of the metabolizable energy, is somewhat less at the lower level of feeding (46.0%) than that obtained at the higher level (51.8%). This difference, however, largely disappears when the apparently aberrant results of rat 13 are omitted from the average (compare 48.3% and 49.8%).

These values for the specific dynamic effect of casein are appreciably higher than the value (31.4%) obtained in a previous experiment by Kriss, Forbes and Miller ('34), when 2 gm. of casein were added to a basal maintenance ration. In the investigation just cited, however, the subjects (rats) were much younger than in the present one. It is possible, therefore, that the difference between the specific dynamic values of casein as determined in the two experiments is at least partly due to the influence of age on the utilization of the nutrient.

When expressed per 100 calories of extra protein katabolized, the specific dynamic effect of casein (average for all rats) is considerably greater at the higher level (69.4 calories) than at the lower level (52.8 calories). If the results of rat 13 be omitted, the average dynamic values would be 55.5 calories and 66.6 calories, per 100 calories of the protein katabolized at the lower and the higher levels, respectively. Although this difference is of appreciable magnitude it can hardly be considered significant in view of the results obtained with the other protein supplements.

Gelatin, on the contrary, shows a somewhat higher specific dynamic effect at the lower level than at the higher level of feeding. The differences are, however, not large. The average specific dynamic effect per 100 calories of extra protein katabolized, for all animals, is 50.4 calories at the lower level, as compared with 47.8 calories at the higher level. The corresponding averages for seven rats are 47.3 and 49.3 calories, respectively. Expressed as per cent of the metabolizable energy, the average specific dynamic effect for the seven animals is 38.2%, at the lower level, as compared with 35.4% at the higher level.

The specific dynamic effect of the heart muscle, expressed either as per cent of the metabolizable energy, or per 100 calories of protein katabolized, is almost identical at the two planes of nutrition. On the basis of metabolizable energy the dynamic values at the lower and the higher levels of feeding are 26.4 and 25.4%, respectively. The dynamic values per

100 calories of extra protein katabolized, at the two stated planes are 54.3 and 53.5 calories, respectively.

It will be observed that while the specific dynamic values, expressed as per cent of metabolizable energy, show considerable differences among the different proteins tested, these differences largely disappear when the specific dynamic effects are expressed in relation to the calories of protein katabolized. In other words, a closer correlation is revealed between the dynamic effects of the various proteins fed and the resulting increases in protein katabolism than there is between the dynamic effects of the proteins and their total metabolizable energy (including body gain). It is possible, however, that these relationships were somewhat affected by the different fat content of the protein supplements (see table 1).

Of the three protein supplements studied casein shows the greatest and heart muscle shows the smallest specific dynamic effect when this is expressed as per cent of the metabolizable energy. The heart muscle as used contained an appreciable amount (9.32%) of lipid material, but its nitrogen content was identical with that of the casein. However, the total increases in heat production caused by the heart muscle at both levels (1944 and 3600 calories) are considerably smaller than the corresponding heat increments of casein (2904 and 6672 calories). It is clear, therefore, that the comparatively low dynamic value obtained for heart muscle, when expressed as a percentage of the metabolizable energy, may be due only in part to its fat content. Another factor which appears to be prominently associated with this low dynamic value is the relatively greater retention of nitrogen in the body (and smaller urinary nitrogen excretion) caused by the heart muscle preparation.

The data for nitrogen retention given in table 4 are based on the previous experiments by Kriss and Voris ('37). Of the total nitrogen ingested as heart muscle 29.1% and 28.6%, were found to be retained in the body at the low and the high levels, respectively, as compared with 13.9 and 21.5% for gelatin, and 6.0 and 17.2%, for casein. The greatest utilization of nitrogen of heart muscle is expected in view of the

relatively complete nature of this protein as compared with the other two. The somewhat greater deposit of nitrogen caused by gelatin, as compared with casein, was not expected, but was apparently effected through the combination with the protein of the basal ration. The relatively high specific dynamic effect of casein, expressed as per cent of the metabolizable energy, is correlated, therefore, with the smallest retention of nitrogen in the body.

The inverse relationship observed between the nitrogen retention in the body, caused by the protein supplements, and their specific dynamic effects, expressed as percentages of their metabolizable energy, together with the relatively closer correlation shown between the increases in heat production and the increases in protein katabolism, lend support to the belief held by Rubner ('02) that protein deposited exerts little, if any, specific dynamic effect.

On the other hand, the results representing the dynamic effects of the protein supplements in relation to the protein katabolized are quite in accord with the findings of Rapport ('24) that casein, gelatin and beef protein have approximately the same specific dynamic action. Obviously, much depends on the way the specific dynamic effects are expressed. In the experiments of Rapport, amounts of the various protein materials with equal content of nitrogen were fed to a fasting dog, and the specific dynamic effects were expressed as per cent of the basal metabolism. There is therefore no basis for quantitative comparisons of our results with those of Rapport.

From the point of view of the present study the most important observation is that, however the results be expressed, the plane of protein intake shows hardly any significant influence on the specific dynamic values of the nutrients, the total protein content of the protein-supplemented rations varying from 31.4 to 45.0%.

In the last two columns of table 4 are presented the net energy values of the protein supplements. In the last column are given the average results for seven rats, while the column preceding the last one contains the averages for all eight rats.

The net energy of the supplements was calculated, in the usual manner, by subtracting from the metabolizable energy of the daily supplement the calories representing the dynamic effect per 24 hours. The results are expressed in calories per gram.

The net energy values of the supplements are, as expected, in inverse relation to their specific dynamic effects, casein having the lowest and heart muscle having the highest net energy value. The comparatively high net energy value of the heart muscle is undoubtedly also due in part to the relatively greater metabolizability of this substance which in turn may be partly attributed to its lipid content.

All three protein supplements show close agreement between their respective net energy values determined at the two planes of nutrition.

The results indicate that within a certain range above the maintenance plane of nutrition the specific dynamic effects and net energy values of proteins are not significantly affected by the quantity of the protein fed, and that the heat production of energy and nitrogen equilibrium may be used satisfactorily as a base value.

Further investigation is necessary to determine definitely whether the same principle applies to other nutrients and to mixed rations.

SUMMARY AND CONCLUSIONS

The specific dynamic effects of dried heart muscle, casein and gelatin were determined with rats when each of these protein materials was given as a supplement to a basal maintenance ration in quantities of 1.5 gm. and 3.0 gm. per day, the heat production of the animals while on the maintenance ration being used as the base value.

Of the three protein supplements tested casein showed the greatest and heart muscle showed the smallest dynamic effect when this was expressed either as total calories or as a percentage of the metabolizable energy. Accordingly, the net energy value of heart muscle was the greatest and that of casein was the smallest.

An inverse relation was observed between the nitrogen retention in the body, caused by the protein supplements, and their dynamic effects expressed as percentages of their metabolizable energy. The results support Rubner's belief that deposited protein has little, if any, specific dynamic effect.

No significant differences were observed between the specific dynamic values of the proteins when fed in the different quantities.

The net energy values of the protein supplements were practically identical at the two different planes of feeding.

The results indicate that the specific dynamic effects of proteins may be satisfactorily determined within a certain range above the maintenance plane of nutrition with the heat production of energy and nitrogen equilibrium as the base value.

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THE EFFECT OF FORCED AIR CURRENTS AND CLOTHING ON RADIATION AND CONVECTION ¹

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THREE FIGURES

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The technic of separating radiation from conduction has been described by Hardy and Du Bois ('38 a); and the results of the basal experiments have been published in the previous paper ('38 b). Practically all of the basal hours were followed immediately by some superimposed factor such as exercise or an electric fan and the resulting changes in heat production and heat loss compared with the basal control.

Barr and Du Bois ('18) after studying malarial chills in the Sage calorimeter asked the following questions.

First, how is it possible that two individuals of the same size can eliminate the same amount of heat if one has a cool skin and the other a warm skin? Second, how can one explain the fact that the heat elimination of a malarial patient remains almost exactly the same per hour during the period before the chill, during the period of chill and during the period of high continuous temperature immediately after the chill?

They themselves explained this by means of a diagram showing with a warm skin the greatest cooling of the blood near the skin. In the case of the cool skin they indicated that the greatest cooling of blood took place at a distance of perhaps 2 to 3 cm. below the skin. Their explanation has been questioned by several writers and their statement of facts has been doubted since it apparently conflicted with Newton's law of cooling.

¹ Clinical Calorimetry, no. 51.

This paradox puzzled us for many years and finally we realized that it could be solved only by a quantitative study of the factors involved in heat loss under various conditions.

The most striking factor studied was the effect of air movement. A large electric oscillating fan was placed at the end of the calorimeter in the corner next to the subject's right foot. The oscillations, at the rate of 9 per minute, blew the air first along the man's right side and then diagonally across his body. The velocity of the air movement over the skin surface could not be measured because of the great amount of turbulence caused by the repeated reflection of the air stream from the walls and sides of the box. At the lowest temperature, 27.4°C., the fan which was producing 16.13 cal. per hour made the skin uncomfortably cool for the first 10 minutes and then gradually became less uncomfortable. At 29°C. there was no discomfort even though the fan was going much faster, producing 36.5 cal. per hour. In the experiments at 31.7°C. and 32.2°C. there was slight sweating in the axillae but no discomfort. At 34.0°C. and 34.7°C. there was slight sweating over the upper parts of the bodies and the least extra exertion, such as taking surface temperature measurements, produced generalized sweating. The fan at these temperatures made the men feel a little more comfortable or rather a little less uncomfortable. It must be remembered that the men were naked and exposed to humidities of 25 to 50%.

The first surface temperature measurements were made while undressing, the second in the preliminary period, 10 to 15 minutes before the start of the respiration experiment. The first hour was basal with no fan or temperature measurements. In the second or transition period of 30 to 45 minutes, the surface temperature was measured in twenty places, then the fan was started and the surface measured again. In the third period, 1 hour in length, the surface was measured once or twice. The last measurements were made after the end of the third period. The fan was still running. The data for six experiments on the two subjects are given in table 1.

TABLE 1
Experimental data on experiments with electric fan

SUBJECT, DATE, WEIGHT, REMARKS	END OF PERIOD	CO ₂	O ₂	H ₂ O	URINARY N PER HOUR	PULSE RATE	TOTAL HEAT PRODUCED	CALORI- METER TEMPER- TURE	CALORI- METER TEMPER- TURE	HUMIDITY	RECTAL TEMPERA- TURE	TOTAL HEAT RATED	SKIN TEMPERATURE		RADIATION	CONVECTION	% VAPOR- IZATION
													Time	Temp- ature			
EFDB. March 13, 1935, 74.7 kg. 1st period basal; 2nd period quiet with electric fan running	11.15	gm.	20.7	0.82	35.4	54	Cal.	68.7	27.38	%	37.25	Cel.	10.56	32.8	%	15	27
	12.15	23.2	20.7	0.82	35.4	54	68.7	27.38	25	37.10	77.4	33.0	11.18	33.0	58	15	27
	1.15	25.9	24.2	0.78	39.5	50	79.7	27.48	25	37.03	99.2	32.8	12.18	32.8	40	37	23
EFDB. May 15, 1935, 77.3 kg. 1st period basal; 2nd period moving, electric fan; 3rd period, quiet, electric fan. * Calc. from CO ₂	11.07										37.26		10.50	33.9			
	12.07	22.6	16.2	1.01	36.7	55	66.4*	28.82	24	37.22	71.2	33.7	12.12	33.7	59	11	30
	12.07	17.2	18.3	0.68	24.7	52	59.5	29.05	24	37.15	58.6	33.6	12.35	33.6	42	31	27
	1.43	23.6	20.5	0.84	36.4	49	63.5	29.40	24	37.15	80.6	32.9	11.15	32.9	41	33	26
											147	33.3	147	33.3			
EFDB. May 22, 1937, 76.8 kg. 1st period basal; 2nd period moving, fan on; 3rd period quiet, fan on	10.41					56	68.9	30.42	29	37.20	72.4	34.0	10.27	34.0	44	25	31
	11.41	23.7	20.6	0.84	38.9	53	45.6	30.54	29	37.14	49.7	34.0	11.45	34.0	36	33	31
	12.16	16.4	13.5	0.89	26.4	53	74.3	30.59	29	37.17	82.8	33.7	12.07	34.0	31	40	29
	1.16	25.6	22.4	0.83	40.4	53				37.19		33.7	12.43	33.7			
JDH. March 4, 1937, 67.1 kg. 1st period basal; 2nd period fan started; 3rd period quiet, fan going	10.44					66	62.5	31.47	28	37.00	63.8	34.8	10.27	34.9	39	20	41
	11.44	20.5	18.8	0.79	44.9	64	52.1	31.67	27	36.98	55.3	34.3	12.00	34.8	30	24	46
	12.32	19.5	15.1	0.94	43.3	64	70.4	31.77	29	37.02	76.3	34.4	12.37	34.4	24	28	46
	1.32	23.9	21.0	0.83	60.7	64				37.03		34.1	14.83	34.1			
EFDB. May 29, 1935, 76.3 kg. 1st period basal; 2nd period moving, fan started; 3rd period quiet, fan going	10.55					57	66.9	32.20	34	37.44	69.4	35.2	10.23	34.9	35	12	51
	11.55	24.0	19.7	0.89	60.0	55	47.5	32.25	34	37.33	56.0	34.8	12.00	35.2	26	18	56
	12.35	17.1	13.9	0.89	53.8	55	69.3	32.28	32	37.29	81.9	34.7	12.40	34.7	27	23	50
	1.35	24.3	20.7	0.86	68.7	55						35.1	14.40	35.1			
JDH. March 9, 1936, 67.3 kg. 1st period basal; 2nd period quiet, fan started; 3rd period, restless, fan going	11.02					66	58.4	33.98	40	36.96	67.3	35.4	10.42	35.4	14	14	72
	12.02	21.1	17.2	0.90	82.7	64	47.3	33.95	42	36.97	54.1	34.8	12.15	35.1	9	4	87
	12.44	16.6	14.0	0.86	81.0	66	63.7	34.00	45	37.02	70.6	34.8	12.55	34.8	9	9	82
	1.44	22.3	18.9	0.86	99.3	66						34.9	1.55	34.9			
EFDB. June 3, 1935, 76.0 kg. 1st period basal; 2nd period quiet, fan started; 3rd period quiet, fan going	11.02					59	67.3	34.73	44	37.41	73.5	35.4	10.36	35.4	5	7	88
	12.02	24.1	20.0	0.88	110.2	57	42.9	34.62	46	37.40	47.1	35.2	12.06	35.2	4	5	91
	12.37	15.9	12.6	0.91	71.1	58	69.5	34.63	49	37.33	80.0	35.3	12.40	35.3	4	5	91
	1.37	24.4	20.8	0.85	124.0	58				37.42		35.5	1.06	35.5			
													1.38	35.5			

The experiments are arranged in order of increasing calorimeter temperature. The first basal periods have been considered in detail and are included here as control periods for the fan experiments. In all except the first experiment a transitional period of about 35 minutes was interposed between the basal hour and the fan hour. This was to allow the fan to be started and the calorimeter to come into gaseous and thermal equilibrium. The rises in heat production seen during the first fan periods are artifacts and are due to blowing off of adsorbed gases on the calorimeter walls. As soon as equilibrium is re-established with the fan running the measurement of heat production is again valid, and the fan is kept running, once it is started, until the subject is removed from the calorimeter. The transition periods are included in order to follow the immediate transitory effects of the fan.

The data are summarized in figure 1 and show that the fan had little or no effect in changing the total metabolism. The moderate increases of 2 to 10 calories per hour in the third period could be accounted for by the slight exertion necessary to make the surface measurements. The rectal temperature showed no effect of the fan except in the experiment at 27.4°C. when there was a temporary fall of 0.2°C. The skin temperature in this same experiment fell almost 1°. In the range between 28.8°C. and 32.2°C. the skin temperature fell about $\frac{1}{2}$ °. At 34.0°C. and above there was the slight drop of 0.3°C. when the fan was started. At 34.7°C. the skin temperature continued to rise in spite of the fan, although a transitory cooling for about 10 minutes was observed. In all experiments the skin temperature showed an immediate fall after the fan was turned on. This was due to the blowing off of the excess moisture on the skin surface. This was followed shortly by a rise in surface temperature to a higher level where the increased air motion played only a relatively small role in the heat loss, affecting neither the convection nor vaporization rates. Air conditioning engineers have long recognized the desirability of intermittent air currents.

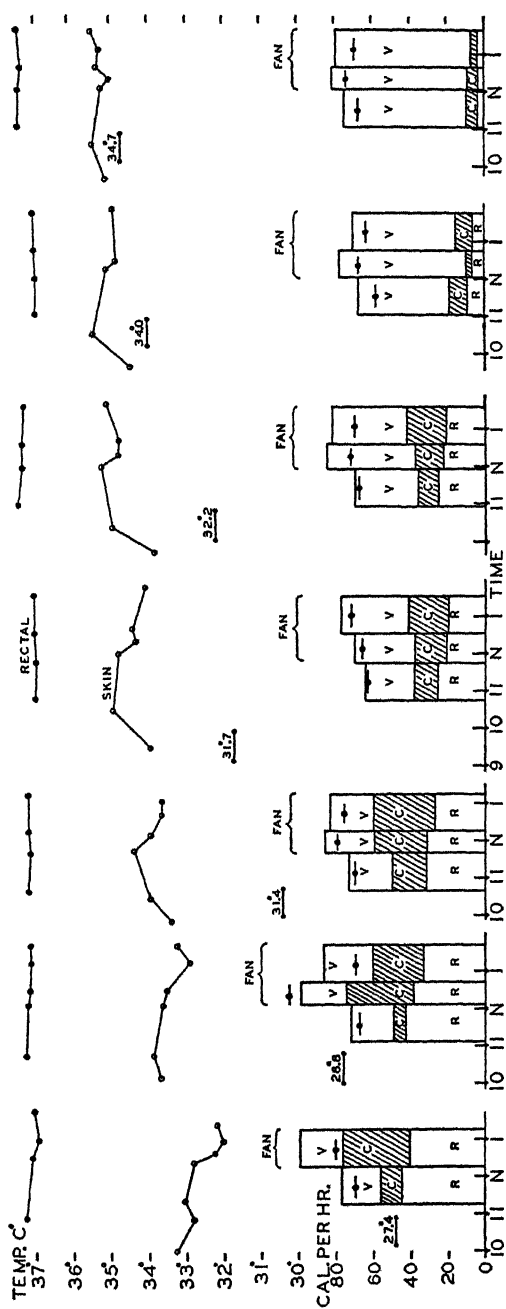


Fig. 1 The effect of forced air currents from an electric fan on metabolism, heat loss, rectal temperature, skin temperature, radiation, vaporization, and convection.

Heat lost by radiation decreased steadily in amount with increasing air temperatures and practically disappeared at 34.7°C. In each individual experiment the cooling of the skin necessarily caused a decrease in radiation. Convection was markedly increased by the fan in the range between 27.4°C. and 30.4°C. until it accounted for 33% to 40% of the total heat lost. At 31.7°C. and 32.2°C. the fan caused only a slight increase in convection, and at 34.0°C. and 34.7°C. convection was even slightly decreased.

Vaporization began to assume an important role in heat loss at 31.7°C., and at temperatures of 34.0°C. and above took care of practically all heat elimination. In the temperature range over 28°C. the electric fan increased vaporization although after blowing off the excess moisture the increase was small, about 10%. The humidity was low enough to insure adequate vaporization in the relatively still air of the calorimeter, and there was no need of the fan. In very humid air at the higher temperatures the fan might be of service in renewing the air near the skin.

The two experiments at the highest temperatures are most instructive. It is evident under these conditions that when the air temperature is within 1° of the skin temperature the fan accomplishes little or nothing. There is no change in vaporization, convection, radiation, or total heat elimination. The reports of the two men that the fan made them feel a little less uncomfortable may have been purely psychic association.

These seven experiments with the fan answer the question of Barr and Du Bois ('18). In the first six the skin became cooler in the second and third periods, and radiation decreased, but increased convection more than made up for this loss and the cool skin eliminated more heat than the warm skin.

CLOTHING

From a practical standpoint the study of men clothed or in bed is more important than the study of men naked. It is easy to measure the radiation from clothing and bedding

and most clothing materials are good 'black body' radiators in the infra-red spectrum from 5μ to 20μ . Unfortunately technical difficulties are greatly increased especially in studying large amounts of bedding because thermal equilibrium is established so slowly. The bedding also prevents free circulation of air in the calorimeter so that the calorimeter values of the heat eliminated may be doubtful. For the above reasons two experiments made on a man with bedding were discarded and our attention turned to rather simple types of closely fitting clothing.

The experimental data of three experiments on subject D are given in table 2. The best results were obtained when the subject D was dressed in ski suits. One of these was the common type of blue woolen material of moderate thickness and of such soft texture that snow clings to it. The jacket, closed with a zipper, fitted snugly and the trousers rather roomy at the hips and knees were tight at the ankles. Underneath the ski suit the man wore thin cotton drawers which came down to the ankles and a thin cotton undershirt with long sleeves. His feet were covered with a pair of thin cotton socks and over these thick woolen ski socks weighing 175 gm. The underclothing weighed 405 gm., the ski suit 1860 gm. The effective radiating surface of the ski suit, feet and head was 1.78 sq.m. and the effective radiating area of the naked man was 1.54 sq.m.

One experiment with exercise was performed in a white cotton suit cut just like a ski suit. It was the type used by track athletes to keep warm before they strip for competition and was made of rather thick material smooth on the outside, fluffy on the inside. The suit weighed 660 gm. The socks and underclothing were the same as those used with the blue ski suit.

The pajamas used in the experiment on February 13, 1935, weighed about 200 gm. A thin sleeveless cotton undershirt was worn under this and the feet were covered with woolen socks.

TABLE 2
Experimental data on clothing experiments

SUBJECT, DATE, WEIGHT, REMARKS	END OF PERIOD	O ₂ gm.	O ₂ gm.	H ₂ O gm.	URINARY N. PER HOUR	PULSE RATE	TOTAL HEAT PRODUCED Cal.	CALORIMETER TEMPERATURE	CALORIMETER HUMIDITY %	RECTAL TEMPERATURE	TOTAL HEAT ELIMINATED Cal.	SKIN TEMPERATURE		RADIATION %	CONVECTION %	VAPORIZATION %
												Time	Temperature			
EFDB. April 2, 1937, 76.8 kg. Cotton ski suit. 1st period basal; last 2 periods mild exercise	10.40									36.85	Cal.	10.22	28.9	67	8	25
	11.40	23.2	20.1	0.84	34.9	60	67.3	22.41	30	36.70	81.8	11.45	28.8			
	12.30	33.4	28.8	0.84	33.4	54	97.1	22.62	33	36.84	83.9	11.50	31.3	67	10	23
	1.05	25.9	23.0	0.82	24.7	53	77.0	22.90	37	37.06	62.6	12.33	29.3	49	28	23
												1.10	29.0			
EFDB. March 12, 1936, 76.5 kg. Wool ski suit and wool socks. Both periods basal	10.30											10.00	28.2			
	11.30	22.6	19.7	0.83	38.5	55	65.7	22.30	37	36.70	83.9	11.34	28.3	66	7	27
	12.30	23.2	20.1	0.84	37.3	51	67.3	22.28	35	36.64	84.0	12.32	28.0	65	9	26
EFDB. February 13, 1935, 76.1 kg. Pejamas. Both periods basal	11.44									37.20		10.48	29.3			
	12.44	22.7	20.6	0.80	32.1	55	68.1	25.09	26	37.14	76.8	1.47	30.1	53	23	24
	1.44	23.5	20.5	0.84	33.2	52	68.3	25.08	26	37.02	75.8			54	20	26

In spite of the difficulties of technic much can be learned by comparing clothed experiments with naked experiments at the same environmental temperature. Subject D dressed in

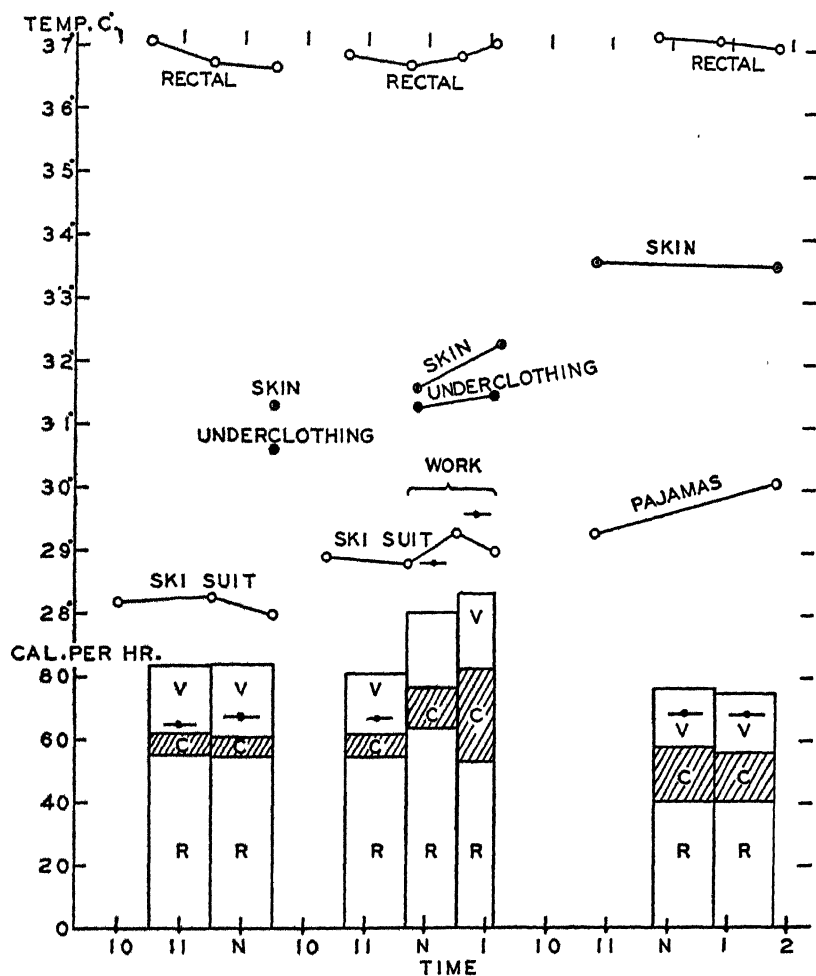


Fig. 2 Heat loss from a clothed subject.

the blue ski suit lay motionless 3 hours in the calorimeter at 22.3. During the first experimental hour he was comfortable but was a little cool the second hour even though the air was 72°F. Just a week before he had been perfectly warm skiing

in exactly this same clothing at a temperature of 16° below zero F. ($-29^{\circ}\text{C}.$). His skin at the end of the calorimeter experiment had an average temperature of $31.2^{\circ}\text{C}.$, about a degree above the shivering point; the surface of the suit was $28^{\circ}\text{C}.$ This same subject naked in the calorimeter experiment at $22.9^{\circ}\text{C}.$ had started with a skin temperature of $31.7^{\circ}\text{C}.$ but it fell rapidly to about $29.9^{\circ}\text{C}.$ when he began to shiver. This same man in experiment with the air $23.4^{\circ}\text{C}.$ had a chill with the skin at $30.1^{\circ}\text{C}.$ The other subject, J.D.H., at similar temperatures had a chill when the skin dropped to $30.7^{\circ}\text{C}.$ The blue and white ski suits slowed the fall in skin temperature. But it is evident that the man would not have been sufficiently protected to prevent the eventual onset of chill.

The pajamas experiment at $25^{\circ}\text{C}.$ with a skin temperature of $33.5^{\circ}\text{C}.$ and surface of pajamas temperature of 29° to 30° may be compared with the experiment on the same man (D) naked at the same temperature when his skin temperature dropped to $31.75^{\circ}\text{C}.$ and he had a chill and with the experiment in which he felt chilly with a skin temperature at $31.8^{\circ}\text{C}.$ The other experimental subject, H., naked at $25.1^{\circ}\text{C}.$ and $25.4^{\circ}\text{C}.$, was chilly when the skin dropped to $32.3^{\circ}\text{C}.$ and $31.7^{\circ}\text{C}.$ The subject with the pajamas felt entirely comfortable, the skin and rectal temperatures remained up and the body was losing heat only very slowly. Thus the cotton pajamas at $25^{\circ}\text{C}.$ offer more protection than the all wool ski suit at $23^{\circ}\text{C}.$

The lines showing the temperature on the surface of the outer clothing, on the surface of the under clothing, and on the surface of the skin give an idea of the gradient from skin to outer air. There is but little radiation from skin to underclothing, more from underclothing to the inside of the outer suit, and still more from the surface of the clothing to the walls of the calorimeter. The effective radiating surface of the ski suits is about 16% greater than that of the naked body. In the experiment on D, naked at $22.9^{\circ}\text{C}.$, the difference between the temperature of the skin and calorimeter walls was

7.0°C. The surface of the ski suit had an average temperature of 28°C. and the thermal difference was 6°C. or 15% less than with the naked man, and this almost exactly balances the greater surface of the clothing. The total radiation thus remains the same under these conditions, with or without clothing. Vaporization at these temperatures is but little changed by ordinary clothing which is porous enough to permit the free passage of water vapor. Convection is not

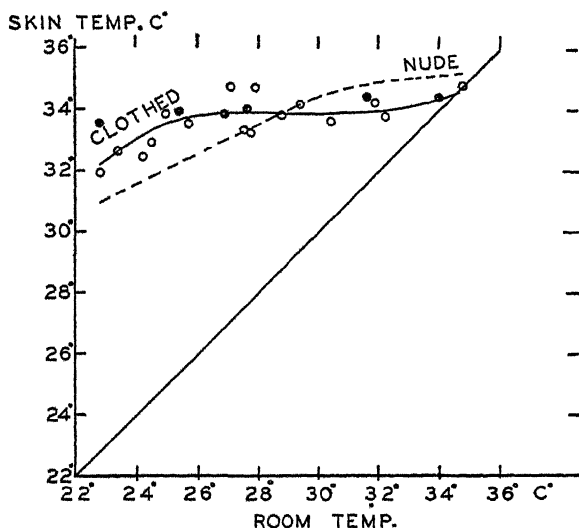


Fig. 3 Effect of clothing upon skin temperature. Circles show average skin temperature under ordinary indoor clothing after the subjects had been sitting quietly for an hour in the prevailing atmosphere. Open circles subject D, solid circles subject II. Dashed line average skin temperature of nude subjects after 2 hours exposure under basal conditions.

greatly changed in quiet experiments with clothing. Motion in the single work experiment caused a great increase in convection just as it does with naked men.

On comparing experiments with the man naked and clothed it appears that when clothing is used the skin corresponds to the subcutaneous tissue of the naked man and loses heat to the outside air through the clothing just as the subcutaneous tissue of the naked man loses heat through the skin. A comparison of the skin temperature under clothing and exposed

is interesting. The subjects always remained at least an hour, before starting an experiment, in ordinary clothing in the prevailing experimental atmosphere. The skin temperature under the clothing was measured as they undressed for the experiment. In the cooler portion of the neutral zone, 28°C. to 30°C., clothing does not affect the skin temperature appreciably, and the skin temperature under the clothing maintains this level (33.8°C.) from 25°C. to 32°C. Thus, in environments lower than 29°C. clothing delays the loss of body heat and does not permit the skin temperature to fall to a chill level. In warmer environments the skin under the clothing was always cooler than the exposed skin. This is a natural consequence of the greater demand placed on the sweat mechanism by the clothed body to help eliminate body heat. The two skin temperature curves cross at about 28.5°C. and this temperature may be taken as the beginning of sensible perspiration in the clothed subjects. Sensible perspiration in the two nude subjects began at about 30°C.

SUMMARY AND CONCLUSIONS

Seven observations were made on the effect of an electric fan blowing over naked men in a calorimeter. There was no significant change in basal metabolism or rectal temperature. With air cooler than 31°C. there was little change in vaporization and radiation but a marked rise in convection which accounted for as much as 33% to 40% of the total heat elimination. In warmer air convection rose less markedly, and in air above 34°C. did not increase at all. At the temperature of 34.7°C. the fan made no change in heat production, heat elimination, radiation, convection, vaporization, rectal temperature, or average surface temperature. It is obvious that with air as warm as the skin (34°C. to 35°C.) and moderate humidity there is no physical benefit to be derived from a fan.

At lower temperatures the fan caused an increase in convection which permitted the body to lose more heat through a cool skin than it had lost in quiet air with a warmer skin.

The effect of clothing was studied in three experiments. Observations made on the clothed man were compared with observations at the same temperature when naked. The surface of the clothes was 1° or 2°C. cooler than the naked skin, but the radiating surface was so much larger that the total radiation was about the same. Vaporization was not changed significantly. The temperature gradient shows a slight drop between underclothing and outer suit, and a still greater drop between the surface of the suit and the walls of the room or the calorimeter.

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SKIN AND BODY TEMPERATURES OF NORMAL INDIVIDUALS UNDER COLD CONDITIONS ¹

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TWO FIGURES

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As a part of an investigation of various physiological processes in schizophrenia, the reaction of the heat-regulating mechanisms to environmental temperature changes has been studied in both schizophrenic and normal subjects. The present investigation deals with the effects on skin and body temperatures of exposure to cold environmental conditions in normal individuals.

The experiment was performed in a psychrometric laboratory in which environmental conditions could be satisfactorily controlled. The skin and body temperatures were measured by thermocouples. The subjects were all studied in the nude under basal conditions, the beds on which they lay being constructed of coarsely woven cane, devoid of any coverings. The skin temperature was measured at nine points, the forehead and eight other areas on the right anterior half of the body surface, namely, the chest, abdomen, upper arm, lower arm, thenar eminence, middle finger, thigh, lower leg, and dorsum of foot. In view of the high degree of similarity of symmetrically located points on the skin (Freeman, Linder and Nickerson, '37), it was felt that an adequate description of the skin temperature could be secured by a study of only one side. Readings were taken

¹ This work was aided by a grant from the Rockefeller Foundation.

30 minutes after recumbency and at three half-hourly periods thereafter, so that the total exposure to the environmental conditions lasted 2 hours.

The effects of two environmental conditions were investigated, viz., 20°C. and 15°C. Ten normal subjects were studied at each temperature level. The figures relating to the levels of temperature and humidity attained during the study are listed in table 1. At the lower temperature, although it was endeavored to make the 5°C. difference the only new variable introduced, significant differences were obtained between the average values and variation of relative humidity and air velocity as well as increased variation in temperature. Such differences may have affected the results to some degree.

TABLE 1

Values pertaining to the environmental conditions attained on the two experimental days (20°C. and 15°C.)

	20°C.		15°C.	
	Mean	Standard deviation	Mean	Standard deviation
Temperature (°C.)	20.13	0.24	15.04	0.44
Relative humidity (%)	21.9	1.56	27.1	2.96
Air velocity (ft./min.)	6.6	4.5	4.3	2.6

The effects of exposure to the experimental conditions upon the levels of skin and rectal temperatures are shown in figure 1. In the case of the skin there is a fall in temperature at almost every location considered. This drop is more marked in the first hour of the experimental day, indicating that some degree of equilibrium is being achieved during the second hour. The case of the forehead is interesting in that the second reading, 1 hour after exposure, is actually higher than that after 30 minutes of exposure, and not until the next reading 90 minutes after exposure is a fall in temperature evident. This initial rise is probably due to the redistribution of blood incident upon changing from the vertical to the horizontal position, the resulting increase in vascularity counteracting the loss of heat to the colder environment and preventing for some time a fall in skin temperature. The same phenomenon has been noted by

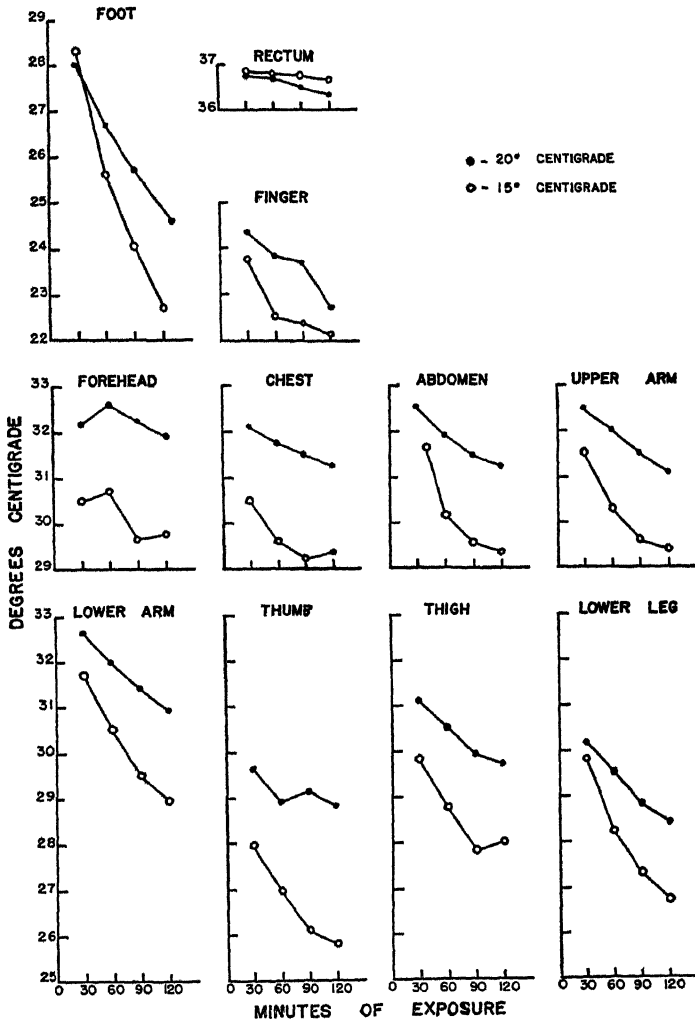


Fig.1 Graphs illustrating the behavior of the averages of the skin and the rectal temperatures in ten normal subjects at 20°C. and the same number of individuals at 15°C. Four readings were taken at intervals of 30 minutes during a total exposure of 2 hours.

Talbot ('31). The different portions of the body surface cool to a varying degree, the drop in temperature over the 2 hours being greatest in the extremities, particularly the feet, where the change from the initial level is approximately $3.5^{\circ}\text{C}.$ at an environmental temperature of $20^{\circ}\text{C}.$ and $5.5^{\circ}\text{C}.$ at an environmental temperature of $15^{\circ}\text{C}.$ The initial fall is much more precipitous at $15^{\circ}\text{C}.$, the drop being of such a degree that a tendency for the establishment of an equilibrium is frequently evident sooner than at $20^{\circ}\text{C}.$, where the rate of heat loss is more gradual. The lower initial temperature of the fingers as compared with that of the feet is probably due to the fact that previous to the examination they had been exposed to cool ward temperatures while the feet had been kept warm by blankets or slippers.

The rectal temperature shows little change until the third determination, 90 minutes after exposure, the trend becoming more marked at the next reading. The drop in rectal temperature over the 2 hours is $0.4^{\circ}\text{C}.$ at $20^{\circ}\text{C}.$, i.e., twice as great as that at $15^{\circ}\text{C}.$ A comparison of the slopes of the rectal temperatures with those of the skin temperatures is of interest. It is apparent that during the first half of the experimental period the skin temperature shows a marked change while the rectal temperature remains relatively unaffected. During the remaining interval, however, the fall in surface temperature becomes less steep while the rectal temperature begins to show a definite drop. In this difference of trends between the internal and external body temperatures it is difficult to evaluate the importance of the vasomotor mechanism as differentiated from the effects of purely physical forces. If the cooling of the organism were influenced solely by physical factors, the delay in change of the rectal temperature might be considered as a lag due to the interposition between the internal and external portions of the body of layers of varying conductivity. This reasoning, however, does not explain why at $15^{\circ}\text{C}.$, with an initial rectal temperature essentially the same as that at $20^{\circ}\text{C}.$, the rectal temperature level falls more slowly than at the higher temperature. It would

seem reasonable that a neurogenic or humoral mechanism must be considered as playing an important role in this reaction.

The vasoconstriction resulting from the cold stimulus decreases the conductivity of the skin and maintains the blood supply in the interior. Heat loss through the skin is thus decreased. Heat production is increased by chemical processes resulting from the discharge of adrenalin. Thus, body temperature is maintained for a time. However, the increased gradient between internal and skin temperatures results in further transfer of heat to the periphery so that heat loss goes on rapidly. As the heat conserving mechanisms fail, the level of body temperature eventually falls and decreases the gradient between it and skin temperature. The diminution in the gradients between the internal and skin temperatures and between the skin and external temperatures results in a slowing of the rate of fall in the skin temperature and the eventual establishment of a state of equilibrium. The lesser fall in body temperature at 15°C. is probably the result both of more intense vasoconstriction and of a greater chemical production of heat. The large surface, lack of adipose tissue, and absence of heat-generating organs, explains the greater fall in temperature of the extremities. In figure 2 are shown the means of the four readings on the ten subjects at each point, measured at both environmental temperatures. From this figure it is evident that the temperatures of the forehead, trunk, and arms hover around the same level; that the temperature of the legs is slightly lower; and that the extremities have the lowest temperature. The legs show a gradient of temperature from the thigh to the foot, while on the upper extremities, the upper and lower arms have the same temperature. The lack of gradation on the arms may be due to their greater proximity to the heart and also possibly to the absorption of heat from the trunk.

In agreement with expectation the average skin temperatures are significantly higher at 20°C. than at 15°C. except for the finger point.

The response of the rectal temperatures, however, to the change in environmental temperature is different from that of the skin temperature. The average level at 15°C. is actually somewhat higher than that at 20°C. although not significantly so.

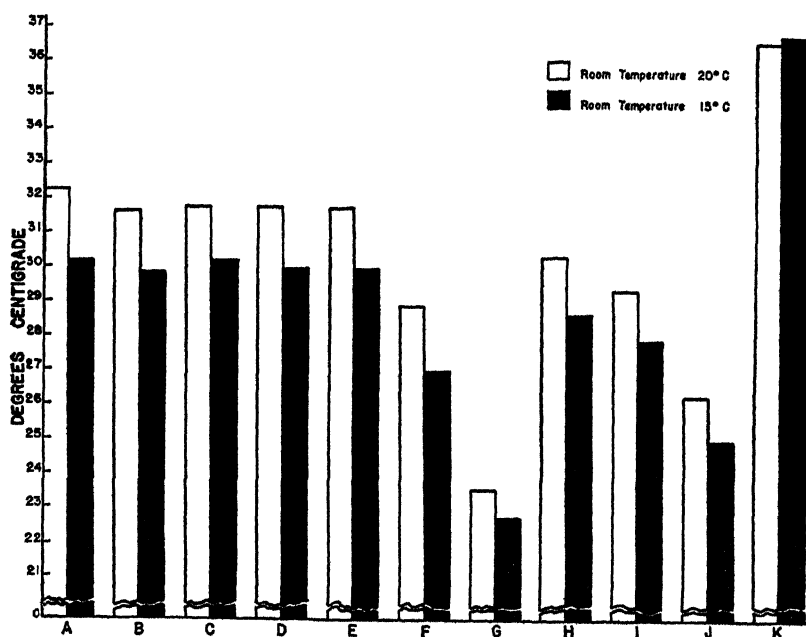


Fig. 2 Averages pertaining to skin and body temperature measurements of four determinations taken at half-hourly intervals over a 2-hour period on each of ten normal subjects at an environmental temperature of 20°C. and the same number of different subjects at 15°C. The letters refer to the points at which the temperature was measured, viz., A, forehead; B, chest; C, abdomen; D, upper arm; E, lower arm; F, thenar eminence; G, middle finger; H, upper leg; I, lower leg; J, foot; K, rectum.

In view of the fact that the averages shown in figure 2 give no information concerning the variation of individuals about them nor as to the change from period to period, analysis of variance has been carried out to determine these.

In table 2 are given standard deviations pertaining to the variation between periods, among individuals, and within individuals. The between period standard deviations illustrate

the variation of the four half-hourly period means around the averages of the group shown in figure 2. Each value is thus a measure of the change in skin or body temperature resulting from exposure to the experimental conditions. As is evident from figure 1, the reaction of the organism is a fall in temperature. Consequently the more pronounced the cooling effect the greater is the variation about a given level of temperature and the higher is the magnitude of the values. The

TABLE 2

Standard deviations of skin and body temperature measurements pertaining to differences between periods, between individuals, and within individuals.

Four determinations on each of ten normal subjects at an environmental temperature of 20°C. and the same number on ten different subjects at 15°C.

SKIN POINT	A ¹		B ¹		C	
	BETWEEN PERIODS		BETWEEN INDIVIDUALS		WITHIN INDIVIDUALS	
	20°C.	15°C.	20°C.	15°C.	20°C.	15°C.
Forehead	0.90	1.66	1.32	2.14	0.50	0.61
Chest	1.14	1.83	0.92	2.11	0.30	0.63
Abdomen	1.82	3.25	1.02	1.89	0.25	0.66
Upper arm	1.92	3.00	0.90	1.46	0.32	0.48
Lower arm	2.22	3.82	1.20	1.31	0.35	0.47
Thumb	2.47	4.50	3.03	2.41	0.90	1.20
Finger	2.17	2.30	3.06	3.13	1.20	1.25
Thigh	1.97	2.92	1.09	2.36	0.35	0.79
Lower leg	2.51	4.25	1.62	1.66	0.28	0.39
Foot	4.53	7.56	3.73	2.02	0.53	0.80
Rectal	0.625	0.272	0.298	0.783	0.138	0.127

¹ Figures in these columns denote significance.

figures in table 2 A are practically all significant when compared with the net intra-individual variation (table 2 C), showing that the cooling effect has been greater than chance variation. As we have seen in figure 1, the forehead is the most constant in temperature while the feet cool to the greatest degree. The more marked fall in temperature at 15°C. is shown by the increased magnitude of the values. The greater stability of the rectal temperature at 15°C. is reflected quite clearly by its lower value.

In table 2 B are shown the standard deviations pertaining to the variation of the individual means about the averages of the group. All these values pertaining to the inter-individual variation are significant when compared with the net intra-individual variation as given in table 2 C. This implies that individuals on the whole have inherently different temperature levels. The values are somewhat larger at 15°C. than at 20°C., implying that decreased temperature increases the difference between individuals.

In general the greatest differences between individuals are found on their extremities. It is of interest that the rectal temperature at 15°C., despite its lesser fall in temperature, shows much greater variation among individuals than it does at 20°C., thus reflecting the trends of the skin temperatures.

The measures of variation obtaining between individuals and between periods yield no information as to the behavior of the individual. Furthermore we need a quantity which may serve as a basis of reference for determining the significance of the preceding measures of variation. Such a quantity is the net intra-individual standard deviation of table 2 C to which reference has already been made. This quantity measures the variation existing, on the average, within the individual after the variation due to cooling has been removed. Thus this net intra-individual standard deviation also measures the extent to which individuals differ from each other in the cooling curves. The greater are the values the greater are the individual differences. The greatest individual differences in cooling curves are found on the hands and, to a lesser degree, on the feet. The values at 15°C. are generally higher than those at 20°C., implying that at the lower temperature there is an increased heterogeneity in behavior.

The data were further analyzed to determine whether any of the following relationships existed: a) between the initial skin temperature and the rate of cooling of the skin; b) between the initial rectal temperature and the rate of cooling of the skin; c) between the average levels of skin and rectal temperatures for the various individuals; d) between the

levels of skin and rectal temperature for a given individual on the average.

No well-marked relationship was observed between the initial temperature of the skin and its rate of cooling. For approximately two-thirds of the locations those individuals having the higher initial skin temperature cooled somewhat more markedly but the relationship appears to be so weak that little emphasis can be laid upon it.

The subjects were divided into two groups having high and low initial rectal temperatures. The cooling curves for the various skin points were also separated on this basis. An analysis of variance performed on the data by this dichotomy did not show any significant relationship. Presumably the initial body temperature cannot serve as an indication of the subsequent course of the skin temperature under the experimental conditions utilized here.

The relationship between the average levels of skin and rectal temperature for the various individuals is very slight and, surprisingly, in a negative direction. Among individuals it averaged -0.13 for both environmental temperatures, and within individuals, -0.24 . The finding of high rectal temperatures with low skin temperatures in normal individuals would imply an active means of maintaining a gradient of heat between the interior and the periphery of the body. While this relationship is ill-defined, it has been shown by other investigators.

Heiser and Cohen ('33) reported a negative relationship between the temperatures of the left wrist and that of the mouth. Steele ('34) noted that the diurnal variation in the temperature of the extremities was opposite to that of the rectal temperature. Burton ('35) found little correlation between the rectal and surface temperatures but in fifteen cases out of forty the changes in these components occurred in opposite directions.

SUMMARY

A study of the skin and rectal temperatures of ten normal subjects exposed to an environmental temperature of 20°C. for 2 hours and an equal number of subjects at a temperature of 15°C. shows the following results:

1. At both environmental temperatures the skin temperatures fall markedly, more rapidly in the first hour and more precipitously at 15°C. than at 20°C. The fall is least on the forehead and greatest on the extremities.

2. The rectal temperature shows little change for an hour but after that begins to fall, more rapidly, however, at 20°C. than at 15°C., the mean level being higher at the latter temperature.

3. Individuals vary from each other significantly as to their temperature levels and differ from each other to the greatest extent on the extremities.

4. The rate of fall in skin temperature is independent of the initial level of the rectal temperature and is only slightly influenced by the initial skin temperature.

5. The average levels of skin and rectal temperatures are slightly related to each other but in a negative direction.

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THE INFLUENCE OF THE DIET AND ENERGY INTAKE UPON ACUTE VITAMIN B₁ DEFICIENCY IN THE RAT ¹

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Although there has been much work reported on the effect of a vitamin B complex deficiency and of a simple vitamin B₁ deficiency in the rat, only scattered reports can be found concerning the actual production of the neuromuscular symptoms associated with acute vitamin B₁ deficiency. Hence it appears that the rat has been found to be more resistant to vitamin B₁ depletion than has the pigeon, the chick, and the dog, as Cowgill ('34) has suggested. In order to determine whether this apparent resistance has been due to the dietary procedure used, a study has been made of the influence of the diet and the method of feeding upon the rat during vitamin B₁ deficiency. Because both the nature of the non-protein constituents of the diet and the method of feeding influenced the results, a study was also made of the energy intake. It is the purpose of this paper to report these results.

EXPERIMENTAL PROCEDURE

The rats came from a Wisconsin strain colony which received the standard stock diet used in this laboratory (Prickett, '34). When started on experiment, they were from 23 to 26 days of age and averaged about 55 gm. in weight. Individual metal cages equipped with false bottoms of hardware

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cloth, having 3 meshes to the inch, were used in all experiments. The animals were weighed weekly and at development of the symptoms of acute vitamin B₁ deficiency or at death. The criterion selected as evidence of acute vitamin B₁ deficiency was the development of neuromuscular symptoms, which have been described in detail by Church ('35) and to a lesser extent by Prickett ('34).

All basal diets contained constant amounts of protein, salt mixture, and agar per Calorie, while the non-protein source of energy was varied (table 1). Three different carbohydrates and one fat were used; the carbohydrates were sucrose, cornstarch, and dextrinized cornstarch, and the fat was coconut fat. In three diets (3 G, 3 B, and 3 D) the carbohydrate furnished all of the energy except that furnished by the protein and the daily supplements. In three other diets (23 C, 23 B, and 23 D) about one-half of the energy from carbohydrate was replaced by fat, and in another single diet about three-fourths of the energy from sucrose was replaced by fat (diet 40 C). In a fourth instance, casein purified by re-precipitation (diet 3 G-1) was compared with the extracted casein (diet 3 G). These caseins were compared in only one diet inasmuch as no great difference was found in the results. The diets were prepared at frequent intervals, and the fat-containing diets were stored at 3°C., except for the short period each day when the rats were being fed.

Two methods of feeding were employed. In one, the rats were fed *ad libitum* after the first 3 days (*ad libitum* group). An attempt was made to feed these rats only such an amount of diet as would be consumed in the succeeding 24 hours. In the other method, the animals were given the same amount of energy (limited group); this was an amount which satisfied the maintenance requirements and allowed a slow rate of growth; after the third day, these rats received 12 + Cal. per day. Any excess diet was weighed back weekly, and upon development of neuromuscular symptoms or death in both groups. In most cases, all rats received the same energy allowance for the first 3 days; namely, 6.25, 9.16, and 9.16 Cal.

for the first, second, and third days, respectively. Such a procedure allowed the animals to become adapted to the diets and thus minimized intestinal disturbances. The energy values of the diets were calculated on the basis of 4.0 Cal. per gram for the carbohydrates, autoclaved yeast, and protein, and of 9.0 Cal. per gram for the coconut fat, cod liver oil, and linseed oil. Besides the rats placed on vitamin B₁ deficient

TABLE 1
Percentage composition of basal diets¹

INGREDIENTS	3 G	3 G-1	3 B	3 D	23 C	23 B	23 D	40 C
Casein (extracted) ²	18.0	...	18.0	18.0	23.0	23.0	23.0	27.0
Casein (reprecipitated) ³	...	18.0
Coconut fat ⁴	23.0	23.0	23.0	40.0
Sucrose ⁵	77.0	77.0	47.5	25.5
Cornstarch ⁶	77.0	47.5
Dextrinized cornstarch ⁷	77.0	47.5	...
Agar	1.0	1.0	1.0	1.0	1.3	1.3	1.3	1.5
Salts 186 ⁸	4.0	4.0	4.0	4.0	5.2	5.2	5.2	6.0

¹ Each rat received 0.25 gm. autoclaved yeast (J. Nutrition, vol. 13, p. 477, '37), 0.1 cc. cod liver oil, and 0.1 cc. raw linseed oil daily as separate supplements.

² J. Nutrition, vol. 8, p. 1, '34.

³ Prepared by twice dissolving in NH₄OH and precipitating with acetic acid. After each precipitation, the casein was washed by percolation with water until the washings were neutral. After the final washing, it was partially dehydrated by washing with 95% alcohol, dried at 40 to 45°C., and ground.

⁴ Pu-Re-Co brand, Capital City Products Co., Columbus, Ohio.

⁵ Commercial granulated sugar.

⁶ Argo brand, Corn Products Refining Co., New York.

⁷ Cornstarch, thoroughly moistened and autoclaved for 5 hours at 15 pounds pressure, dried at 60 to 70°C., and ground.

⁸ J. Biol. Chem., vol. 89, p. 199, '30.

diets, control rats were fed certain of the basal diets and adequate vitamin B₁.

All rats received daily supplements of 0.1 cc. of cod liver oil, 0.1 cc. of raw linseed oil, and 0.25 gm. of autoclaved yeast. These supplements were given in a separate container, except in the limited feeding where the autoclaved yeast was mixed into the basal diets at such a level that each rat would receive 0.25 gm. in the daily food. The control rats received adequate

vitamin B₁ as 20 mg. of a fuller's earth adsorbate of a brewer's yeast extract on alternate days (Salmon and Goodman, '37). Distilled water was always available.

RESULTS

Effectiveness in producing neuromuscular symptoms

The nature of the non-protein constituents had much more influence on the effectiveness of the diets in producing the neuromuscular symptoms of acute vitamin B₁ deficiency than did the method of feeding (table 2). The combined data show that the dextrin and starch diets (3 D and 3 B) were least effective, the sucrose, the dextrin-fat, and the sucrose-high fat diets (3 G, 23 D, and 40 C) were next, and the starch-fat and sucrose-fat diets (23 B and 23 C) were most effective. The percentages of rats developing neuromuscular symptoms were 44, 46, 65, 67, 69, 90, and 93 for diets 3 D, 3 B, 3 G, 23 D, 40 C, 23 B, and 23 C, respectively.

Although fewer rats were subjected to the ad libitum than to the limited method of feeding, the ad libitum procedure appeared to be somewhat more effective in producing the symptoms than did the limited procedure (table 2). This was especially true for diets 3 D, 23 D, and 40 C and may have been caused by the longer time these rats were on experiment. Certainly, other deficiencies, as well as the low level of food intake, would affect the rats adversely in the longer experimental periods.

The sex of the rats did not seem to have any especial influence on the development of acute vitamin B₁ deficiency. A somewhat higher percentage of females (74%) than of males (69%) exhibited neuromuscular symptoms, but it is considered that this difference was not significant. These percentages are based on a total of 223 female and 274 male rats. Patras and Templeton ('33) have reported similar results.

TABLE 2

The development of neuromuscular symptoms and the weight changes of rats in acute vitamin B₁ deficiency as influenced by the diet and method of feeding

SERIES	SUPPLE- MENT	DIET	NUMBER OF RATS		INITIAL WEIGHT		FINAL WEIGHT		MAXIMUM WEIGHT		TIME MAXIMUM WEIGHT ATTAINED		TIME TO	
			Total	Devel- oping N.M.S. ¹	Devel- oping N.M.S. ¹	Death no N.M.S. ¹	Devel- oping N.M.S. ¹	Death no N.M.S. ¹	Devel- oping N.M.S. ¹	Death no N.M.S. ¹	Devel- oping N.M.S. ¹	Death no N.M.S. ¹	N.M.S. ¹	days
Limited (iso- caloric) ²	None	3 G	122	78	58	57	45	40	59	57	14	2	52	43
		3 G-1	18	16	55	53	43	41	59	55	17	14	51	41
		3 B	45	20	57	55	43	38	59	56	12	3	50	43
		3 D	28	7	52	55	49	44	72	62	76	46	119	123
		23 C	101	95	56	58	51	39	60	58	22	0	68	51
		23 B	29	27	57	54	51	46	62	61	25	45	59	79
		23 D	28	17	58	59	50	41	66	62	59	36	129	77
		40 C	15	8	53	55	48	45	58	61	47	61	105	138
		3 G	39	56	..	51 ⁴
		3 B	11	53	..	44 ⁴
Ad libitum	None	23 C	28	56	..	57 ⁴
		3 G	17	12	54	58	44	42	61	62	10	8	41	31
		3 G-1	12	10	55	59	44	40	62	66	11	10	44	41
		3 B	12	6	56	55	49	40	67	61	15	8	44	35
		3 D	17	13	57	54	63	38	82	60	40	10	71	32
		23 C	21	19	55	57	52	46	68	69	19	14	42	46
		23 B	12	10	55	50	58	41	74	66	25	14	49	29
		23 D	12	10	55	54	71	55	84	58	43	3	62	11
		40 C	11	10	57	58	75	92	87	95	69	147	85	171
		3 G	13	57	..	92 ⁴
	Vita- min B ₁ ³	3 B	5	52	..	94 ⁴
		23 C	4	53	..	108 ⁴

¹ The neuromuscular symptoms of acute vitamin B₁ deficiency.

² Each rat received 12+ Cal. per day after the third day.

³ J. Nutrition, vol. 13, p. 477 ('37). Each rat received 20 mg. on alternate days of a fuller's earth adsorbate of a brewer's yeast extract.

⁴ Weight at 42 days.

Time to development of neuromuscular symptoms

On every diet, subjecting the rats to the limited method of feeding increased the time to the onset of neuromuscular symptoms (table 2). This increase in the time was greater for the fat-containing diets (23 C and 23 B) than it was for the high carbohydrate diets (3 G and 3 B), suggesting that this level of coconut fat exerted a 'sparing' effect on vitamin B₁ when the rats were fed by the limited method. The 'sparing' effect of the coconut fat was found in the high fat diet (40 C) in the ad libitum as well as in the limited method of feeding.

A decided increase occurred in the time required for rats to develop neuromuscular symptoms when dextrinized cornstarch (diets 3 D and 23 D) was substituted for the original cornstarch in diets 3 B and 23 B (table 2). Again, the greatest increases in time occurred in the limited feeding procedure. The dextrin appeared to exert about as great a 'sparing' effect on vitamin B₁ as did 40% of coconut fat.

There appeared to be a relation between the effectiveness of a diet in producing the neuromuscular symptoms and the time of appearance of the symptoms; the diets and method of feeding that produced the neuromuscular symptoms in the shortest and longest times were least effective in producing them in a large percentage of individuals, while the diets and method of feeding that produced the symptoms in intermediate times were most effective. Even then there were exceptions, but these occurred mainly in the fat-containing diets and in the ad libitum method of feeding. Thus, although the fat did not exert a 'sparing' effect in the lower level on the ad libitum feeding, it did materially influence the effectiveness of a diet in producing the neuromuscular symptoms of acute vitamin B₁ deficiency.

The inclusion of fat or of dextrin in the diet or the use of the limited feeding method widened the spread of distribution of the rats which developed neuromuscular symptoms; the greatest concentration in the incidence of distribution was nearest to the average incidence of neuromuscular symptoms

on the ad libitum feeding procedure. Even in those diets which were most effective in producing neuromuscular symptoms of acute vitamin B₁ deficiency, there was a considerable spread.

Some work was also done on the relation of autoclaved yeast dosage to the development of neuromuscular symptoms because a previous report from this laboratory (Salmon and Goodman, '37) indicated that, although the yeast was autoclaved moist for 8 hours at 120°C., it still contained traces of vitamin B₁. Three litters of six rats each were fed diet 3 G-1 (table 1); twelve rats received the usual 0.25 gm. of autoclaved yeast daily and six litter mates received 0.5 gm. daily. Ten of the rats on the lower level of yeast developed neuromuscular symptoms in an average of 56 days and four of the rats on the higher level developed symptoms in an average of 68 days. The increase in time to onset of symptoms on the higher level could be considered as evidence supporting the conclusion reached by Salmon and Goodman ('37). This increase in the time by doubling the autoclaved yeast allowance was of about the same degree as was secured by replacing about 50% of the energy from sucrose by that from coconut fat (diet 23 C) on the same (limited) feeding procedure.

Weight changes

Very little difference was found in the maximum weights of the rats that developed neuromuscular symptoms on all diets in the limited feeding procedure, with the exception of the rats which were fed the dextrin diets, 3 D and 23 D (table 2). These rats gained more weight than the rats on the other diets, despite the fact that all rats received the same energy allowance.

Similar but less uniform results were secured from the ad libitum feeding. In most cases, these rats reached a greater maximum weight than the limited fed rats. The rats which received diet 40 C had the greatest maximum weight, but those that were fed diets 3 D and 23 D were not much lighter.

The weight loss (difference between maximum and final weights) of the rats developing neuromuscular symptoms was somewhat greater and more rapid in the ad libitum than in the limited feeding (table 2). The greatest losses in weight occurred on the high carbohydrate diets (3 G, 3 B, and 3 D), and the least losses occurred on the high fat diet (40 C). With the exception of diet 40 C, the diets and method of feeding which induced the most rapid losses in weight were the least effective in producing the neuromuscular symptoms of the acute deficiency.

Growth was essentially the same in the limited feeding method for the rats that received vitamin B₁ as it was for those not receiving the vitamin (table 2). This made it appear that the rats not receiving the vitamin were utilizing the food about as efficiently as were those that received the vitamin supplement. However, it was always observed in the paired, as well as limited and isocaloric, feeding that the animals which received vitamin B₁ could not be reduced to the same level of food intake as could the deficient rats without causing death.

The weight records of the ad libitum fed rats which received vitamin B₁ show that these rats did not grow as rapidly as rats on stock diet (table 2). Even 0.5 gm. of untreated yeast daily per rat did not give normal growth.

Energy intake relations

The energy intake required for the development of the neuromuscular symptoms of acute vitamin B₁ deficiency was markedly influenced by the non-protein constituents of the diet (table 3). Although the energy intake was similar for the high sucrose and high starch diets (3 G and 3 B), partially substituting coconut fat or dextrin for the sucrose or cornstarch materially increased the energy requirement for the production of neuromuscular symptoms. This was especially pronounced when three-fourths of the non-protein energy in the high sucrose diet (3 G) was replaced by coconut fat (diet 40 C), or when the cornstarch in diets 3 B and 23 B was replaced by dextrin (diets 3 D and 23 D).

TABLE 3

The energy intake of rats in vitamin B₁ deficiency as influenced by the diet and method of feeding

SERIES	DIET	NUMBER OF RATS	TIME TO		TOTAL ENERGY		DAILY ENERGY	
			N.M.S. ¹	Death no N.M.S. ²	Allotted	Consumed	Allotted	Consumed
Limited (iso-caloric) ²	3 G	78	days	days	Cal.	Cal.	Cal.	Cal.
	3 G-1	16	51.6	...	613	535	11.87	10.36
	3 B	20	50.6	...	601	522	11.87	10.31
	3 D	7	50.4	...	598	544	11.87	10.79
	23 C	95	118.7	...	1420	1190	11.97	10.02
	23 B	27	67.6	...	805	788	11.91	11.66
	23 D	17	59.3	...	705	681	11.89	11.49
	40 C	8	127.1	...	1522	1388	11.97	10.92
Ad libitum	3 G	12	104.7	...	1252	1227	11.96	11.72
	3 G-1	12	40.6	465	11.45
	3 B	10	43.6	485	11.11
	3 D	6	43.7	583	13.34
	23 C	13	71.4	1415	19.82
	23 B	19	42.1	679	16.14
	23 D	10	49.1	848	17.26
	40 C	10	62.0	1414	22.81
Limited (iso-caloric) ²	3 G	44	84.7	1515	17.77
	3 G-1	2	...	43.5	515	425	11.84	9.77
	3 B	25	...	40.7	481	380	11.83	9.34
	3 D	21	...	43.4	514	433	11.84	9.97
	23 C	6	...	122.6	1467	1360	11.97	11.09
	23 B	2	...	51.5	611	598	11.87	11.60
	23 D	11	...	79.5	948	928	11.93	11.67
	40 C	7	...	77.0	918	895	11.93	11.62
Ad libitum	3 G	5	...	138.3	1656	1605	11.98	11.61
	3 G-1	2	...	31.0	...	437	14.10
	3 B	6	...	41.0	...	423	10.33
	3 D	4	...	35.3	...	422	11.95
	23 C	2	...	32.2	...	551	17.12
	23 B	2	...	46.2	...	684	14.80
	23 D	2	...	29.5	...	418	14.17
	40 C	1	...	11.5	...	286	24.87
Ad libitum	3 G	1	...	171.0	...	3408	19.93

¹ The neuromuscular symptoms of acute vitamin B₁ deficiency.

² Each rat received 12+ Cal. per day after the third day.

The method of feeding influenced the energy intake of the rats that developed neuromuscular symptoms to a lesser extent than the diet (table 3). The greatest difference in caloric intake on the two feeding methods was found on those diets which required the longest time for development of neuromuscular symptoms.

With the exception of diet 40 C, the two diets which were most effective in producing the neuromuscular symptoms of acute vitamin B₁ deficiency in the limited feeding procedure (diets 23 C and 23 B) gave the nearest to the allotted daily energy intake. The diet which was eaten in the least amount (diet 3 D) was the least effective.

Food was consumed more uniformly by the limited (isocalorically) fed than by the ad libitum fed rats, especially in the latter stages of the deficiency; that is, the ad libitum fed rats reduced the food intake much more drastically shortly before the development of neuromuscular symptoms than the limited fed rats. Whereas the limited fed rats were forcibly subjected to a continuous or chronic inanition due to the low level of food intake, the ad libitum fed rats voluntarily subjected themselves to a more acute inanition in the latter stages of the deficiency.

In general, the rats that died without developing the neuromuscular symptoms consumed less total and daily energy than did the rats on the same diet that developed the symptoms of the acute deficiency (table 3). Again the data do not bring out a point that may be significant, namely, that the rats which died without developing neuromuscular symptoms usually reduced their food intake earlier than the rats which did develop neuromuscular symptoms. This was especially true of the rats on diets 3 G and 3 B.

DISCUSSION

These experiments demonstrate that it is possible to produce consistently the neuromuscular symptoms of acute vitamin B₁ deficiency in young rats. On certain diets, at least 90% of the rats developed neuromuscular symptoms. The

most effective diet studied was one in which about one-half of the energy from non-protein sources was furnished by coconut fat and one-half by either sucrose or cornstarch. As a whole, the least effective diets were those in which all of the energy from non-protein constituents was supplied by carbohydrate. This was especially true in the limited method of feeding. These results are essentially in agreement with those of Braddon and Cooper ('14 a, '14 b), and Ariyama ('34).

The time required for the development of neuromuscular symptoms of acute vitamin B₁ deficiency in young rats was influenced by the method of feeding as well as by the nature of the non-protein constituents of the diet. As compared with the results obtained from the ad libitum method of feeding, subjecting the rats to a limited energy allowance increased the time on all diets. Moreover, the diets containing sucrose or cornstarch with one-half of the energy from non-protein sources supplied by coconut fat gave an increase in the time to acute vitamin B₁ deficiency over that obtained from the carbohydrates alone in the limited feeding method, whereas no such effect occurred in the ad libitum feeding method. This result would suggest a 'sparing' action of the fat on vitamin B₁ on the low level of fat when the rats were fed at a low level of energy intake. Replacement of three-fourths of the energy from non-protein sources by coconut fat gave a fat 'sparing' action even in ad libitum feeding. However, the same result was obtained in the diets in which cornstarch was replaced by dextrinized cornstarch; in the ad libitum feeding, the time to acute vitamin B₁ deficiency was increased to about the same extent for the high dextrin diet (3 D) as for the high fat diet (40 C). These results with dextrin seem to be in agreement with those reported by Guerrant et al. (Guerrant and Dutcher, '34 a, b; Guerrant, Dutcher and Tomey, '35; and Guerrant, Dutcher and Brown, '37) on the vitamin B complex.

Postulation of a relationship between the food or energy intake and vitamin B₁ requirement was advanced long before it was known that vitamin B₁ was only one constituent of a complex (Funk, '14; Braddon and Cooper, '14 a, b; Green, '18;

and Plimmer, '26). Recently, added proof that this relationship was actually concerned with the B₁ fraction of the vitamin B complex has been advanced by Amantea ('33, '34, and '35) and by Cowgill ('34). These latter workers have developed formulas, based on the animal weight and food intake, for the determination of the onset of acute vitamin B₁ deficiency or of the vitamin B₁ requirement for growth. The results of the experiments reported in this paper do not deny that there is a direct relationship between the food intake and the production of acute vitamin B₁ deficiency in the young rat, but emphasize that the nature of the diet and the plane of nutrition influence this relationship. Only the non-protein constituents of the diets were varied in these experiments; increasing the fat (coconut fat) up to 40% or substituting dextrinized cornstarch for the original cornstarch more than doubled the energy requirement for the development of neuromuscular symptoms. The plane of nutrition had a less pronounced effect: the energy required for the production of the acute symptoms was similar on certain diets whether the rats were fed ad libitum or at a limited level, whereas on other diets one method of feeding required more energy intake than did the other. These results show, therefore, that any formula devised for calculation of the vitamin B₁ requirement for energy utilization must take into account the source of the energy as well as the plane of nutrition.

SUMMARY AND CONCLUSIONS

1. The effectiveness of a diet in producing the neuromuscular symptoms of acute vitamin B₁ deficiency in the young rat varied decidedly with the nature of the non-protein constituents of the diet, all other components of the diet remaining constant. The most effective diets studied were those in which carbohydrate (sucrose or cornstarch) and fat (coconut fat) each furnished approximately half of the non-protein source of energy. About 90% of the rats on these diets developed neuromuscular symptoms. The method of feeding had less influence than the diet, although the ad libitum method was

somewhat more effective than a limited (isocaloric) method in which each rat received 12+ Cal. per day.

2. The time to onset of neuromuscular symptoms was influenced by both the method of feeding and the nature of the non-protein constituents of the diet. The time was greater for the limited than for the ad libitum feeding method. Replacement of one-half of the energy from carbohydrate (sucrose or cornstarch) by that of coconut fat had no effect in the ad libitum feeding but increased the time in the limited feeding procedure. Replacement of cornstarch by dextrinized cornstarch or of three-fourths of the sucrose in the sucrose diet by coconut fat (energy basis) increased the time in both feeding methods; the effect was as great for dextrin as for the coconut fat.

3. The rats on the limited feeding procedure usually attained a lesser maximum weight and the loss from the maximum to the final weight was less rapid and of a lesser degree than for the rats on the ad libitum feeding procedure. Likewise, anorexia was less acute with the limited method of feeding, especially for those diets which were most effective in producing the neuromuscular symptoms.

4. The nature of the non-protein constituents of the diet and, to a lesser degree, the method of feeding, influenced the total energy required for the production of neuromuscular symptoms. The substitution of dextrinized cornstarch for cornstarch in the high starch diet or of coconut fat for about three-fourths of the energy from sucrose in the high sucrose diet more than doubled the energy intake before the onset of neuromuscular symptoms.

5. If the proper diet is used, the incidence of the neuromuscular symptoms of acute vitamin B₁ deficiency is so consistent in young rats that it is suggested that these symptoms constitute the basic criterion of acute vitamin B₁ deficiency in the rat, as well as in the pigeon and the chick.

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THE QUANTITATIVE REQUIREMENT OF THE GROWING CHICK FOR MANGANESE¹

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Deficiency of manganese in the feed of chicks (Wilgus, Norris and Heuser, '36, '37) results in a leg disorder known as slipped tendon or perosis. This condition is characterized by a bowing of the leg at the tibia-metatarsal joint, enlargement with a tendency toward flattening of the joint and finally slipping of the Achilles tendon from its normal position. Recent investigations (Gallup and Norris, '37 a, '37 b); Heller and Penquite, '37; Schaible, Bandemer and Davidson, '37; Lyons, Insko and Martin, '37; and Insko, Lyons and Martin, '37) have confirmed the earlier work on the necessity of manganese for the prevention of this abnormality. The experiments reported in this paper were designed to determine the level of manganese most satisfactory for growth and for the prevention of perosis.

All lots of chicks were wing banded at random as described by Wilcke. ('36), weighed individually and placed in wire-screen-floored, hot-water-heated battery brooders. The all-mash ration and distilled water were given ad libitum, the water in glazed earthenware fountains. The basal all-mash ration was composed of ground yellow corn 62.5, dried skim-milk 20.0, meat scrap 6.0, liver meal 5.0, soybean oil 3.0,

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sardine oil 1.0, steamed bone meal 2.0, and salt 0.5 parts by weight. This basal all-mash contained 1.56% calcium, 1.03% phosphorus, and 7 ppm. (parts per million) of manganese in the first experiment and 1.67% calcium, 1.01% phosphorus and 6 ppm. of manganese in the second.

All chicks were weighed and observed individually at weekly intervals. The degree of bowing of the legs and the position of the Achilles tendon were determined. The degrees of bowing were recorded as 1, 2, 3 and 3.5 where 1 represented a straight leg (normal) and 3.5° of bowing hardly distinguishable from slipped tendon. The point of the bow is the tibia metatarsal joint, not the diaphysis of the metatarsus.

FIRST EXPERIMENT

Eight lots of day-old Rhode Island Red chicks containing fifty chicks each were started in this experiment. At the end of 1 week thirty-five chicks from each lot were selected for inclusion in the complete experiment. The basal all-mash containing 7 ppm. was supplemented by adding manganese sulfate ($\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$), as follows: lot 1, none; lot 2, 10 ppm. of manganese; lot 3, 20 ppm. of manganese; lot 4, 30 ppm. of manganese; lot 5, 40 ppm. of manganese; lot 6, 60 ppm. of manganese; lot 7, 80 ppm. of manganese; and lot 8, 100 ppm. of manganese. The results of this experiment are shown in table 1.

The quantity of manganese in the ration influenced the percentage of slipped tendons inversely. Seventy-seven per cent of the chicks of lot 1 receiving the basal ration, containing 7 ppm. of manganese, had slipped tendons. Fifty-four per cent of the chicks receiving 17 ppm. of manganese had slipped tendon, while of those receiving 27 ppm. of manganese, about 25% had slipped tendon. Manganese at higher levels entirely prevented slipped tendons, with the exception of one chick in lot 6. This will be discussed later.

The average degree of bowing is also influenced inversely by the amount of manganese in the ration. It is noticeable that at 8 weeks of age as the quantity of manganese increases

there is a lower average degree of bowing. Lot 1 receiving the basal ration (7 ppm. of manganese) had an average degree of bowing of 3.23 while lot 8 receiving 107 ppm. of manganese had an average of 1.36.

The average weight of the males at 8 weeks of age was heaviest in lot 4 which received 37 ppm. of manganese, while the heaviest average weight of the females was in lot 5 receiving 47 ppm. of manganese. The lowest average weight was that of lot 1 which received only the basal ration which

TABLE 1

*Effect of level of manganese intake on growth and incidence of perosis.
First experiment¹*

LOT NO.	MANGANESE PPM.	AVERAGE WEIGHT OF CHICKS, GRAMS		AVERAGE DEGREE OF BOWING		PER CENT OF SLIPPED TENDON	MORTALITY PER CENT
		Male	Female	4 weeks	8 weeks		
1	7	433	442	3.0	3.2	77.1	20.0
2	17	575	576	2.9	2.9	54.3	17.1
3	27	641	560	2.5	2.5	25.7	20.0
4	37	693	582	1.8	1.7	0.0	5.7
5	47	678	621	1.7	1.5	0.0	5.7
6	67	619	562	1.8	1.4	2.9	0.0
7	87	627	556	1.7	1.3	0.0	2.9
8	107	658	605	1.7	1.3	0.0	2.9

¹ Thirty-five chicks selected at end of first week for each lot in experiment. All chicks started in lot in which they continued.

contained 7 ppm. of manganese. When larger quantities of manganese were added to the ration there was not a marked difference in average weight at 8 weeks of age. There was, however, a lower average weight in all lots when the quantity of manganese was above 47 ppm.

The percentage of mortality decreased as the quantity of manganese in the ration was increased. Most of the mortality in the lots in which a high percentage of slipped tendon occurred was caused by inability of the chicks so affected to obtain enough feed.

SECOND EXPERIMENT

Nine lots of Rhode Island Red chicks containing thirty chicks each were used in this experiment. The basal all-mash was supplemented by adding manganese sulfate ($\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$) as follows: lot 11, none; lot 12, 10 ppm. of manganese; lot 13, 20 ppm. of manganese; lot 14, 30 ppm. of manganese; lot 15, 40 ppm. of manganese; lot 16, 80 ppm. of manganese; lot 17, 160 ppm. of manganese; lot 18, 320 ppm. of manganese; lot 19, 640 ppm. of manganese. The results of this experiment are shown in table 2.

TABLE 2

*Effect of level of manganese intake on growth and incidence of perosis.
Second experiment*

LOT NO.	MANGA- NESE PPM.	NUMBER SURVIVING 2 WEEKS	AVERAGE WEIGHT OF CHICKS, GRAMS		AVERAGE DEGREE OF BOWING		PER CENT OF SLIPPED TENDON	MORTALITY PER CENT
			Male	Female	4 weeks	8 weeks		
11	6	24	430	365	3.0	3.1	45.8	36.7
12	16	28	461	428	3.1	3.1	64.3	20.0
13	26	19	584	489	2.7	2.1	21.1	40.0
14	36	27	609	531	1.9	1.7	3.7	10.0
15	46	23	520	543	1.8	1.7	0.0	26.7
16	86	26	616	524	1.8	1.7	0.0	13.3
17	166	28	584	506	1.8	1.4	0.0	10.0
18	326	25	559	568	1.9	1.3	0.0	16.7
19	646	26	629	634	1.7	1.2	0.0	13.3

Since there was a rather high mortality in some lots during the first 2 weeks, the percentage of slipped tendons is based on the number alive at the end of that period. There were no cases of slipping at that time.

The lower quantities of manganese again allowed the incidence of an appreciable percentage of slipped tendons. There was not so large a percentage of slipped tendons in the lot fed the lowest level of manganese as in the first experiment. The degree of bowing of the legs, however, indicates an advanced stage which closely approaches actual slipping of the tendon. The greatest differences in degree of bowing were noted at the eighth week. Degree of bowing was again influenced inversely by the amount of manganese fed the chicks.

This experiment again indicates that at least 30 ppm. of manganese should be added to this basal mash for satisfactory growth of chicks without the incidence of slipped tendon. The quantities of manganese in this experiment which were much higher than in the first trial failed to manifest a toxic effect.

DISCUSSION

In these experiments a minimum of 30 ppm. of manganese added to the basal ration used was necessary for good growth and for the prevention of slipped tendons. These experiments agree in this regard with those reported by Wilgus, Norris and Heuser ('36, '37) and Gallup and Norris ('37 a). The former investigators found that the addition of 0.0025% of manganese to a diet containing 0.0010% of this element was sufficient almost entirely to prevent perosis. The latter investigators observed that occasionally a chick receiving an adequate amount of manganese developed perosis during the first 10 days. The same workers (Gallup and Norris, '37 b) found that the isolated cases developing early in life could not be prevented by adding manganese in high concentrations to the drinking water or to the feed. Results obtained in lot 6 of the first experiment reported herein, in which one chick developed slipped tendons although the mash contained 67 ppm. of manganese, are in agreement with the results reported by Gallup and Norris. The abnormality was apparent at the end of the second week, and the tendon slipped during the fourth week.

Lyons and Insko ('37) fed to laying hens diets which produced a high percentage of slipped tendons in chicks. This treatment greatly reduced hatchability. The embryos which failed to hatch had very short legs, protruding abdomen, 'parrot beak' and bulging head. The addition of manganese to the diet prevented this condition. An experiment just completed indicates that hens on a diet deficient in manganese produce chicks which develop perosis earlier than chicks whose mothers receive a diet containing a satisfactory quantity of manganese.

The experiments reported herein indicate that manganese fed as manganese sulfate ($\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$) at levels as high as 646 ppm. was not toxic. This is in agreement with the work of Gallup and Norris ('37 a) who used several manganese compounds with the same results.

Even though toxic levels of manganese were not reached in these experiments, the results show that there is a wide range between the minimum protective level and the level at which toxic symptoms appear (wide therapeutic index). Heller and Penquite ('37) have shown that a ration containing approximately 4800 ppm. (0.48%) is highly toxic to young growing chicks. The exact minimum protective level of manganese cannot be definitely established until we have a thorough knowledge of the relationship between the calcium and phosphorus content of a ration and the availability of manganese. There are perhaps three main factors which operate to determine the minimum protective level of manganese in the chick's diet: 1) the level of available manganese in the diet of the mother hen; 2) the calcium and phosphorus content of the ration; and 3) the availability of the manganese supplement. However, as stated above, the minimum protective level of manganese under the conditions of the experiments herein reported appears to be near 35 or 40 ppm. of manganese.

SUMMARY

These experiments were designed to determine the level of manganese most satisfactory for growth and for the prevention of perosis. An all-mash ration containing 6 or 7 ppm. of manganese was supplemented with manganese sulfate.

A minimum of 30 ppm. of manganese added to the basal ration used was necessary for good growth and for the prevention of slipped tendons. Lower quantities allowed the incidence of an appreciable percentage of slipped tendons. The degree of bowing of the legs was inversely proportional to the content of manganese in the feed up to about 30 ppm. As much as 646 ppm. was not toxic.

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SUPPLEMENT

PROCEEDINGS OF THE FIFTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

SOUTHERN HOTEL, BALTIMORE, MARCH 30, 1938

The fifth annual meeting of the American Institute of Nutrition was held in Baltimore, at the Southern Hotel on March 30, 1938. One hundred and twelve members and 486 guests registered but there were over 600 in attendance during the scientific session.

COUNCIL MEETING

The Council meeting was held at the Southern Hotel, Tuesday evening, March 29th, 6.30 P.M. All members were present except L. A. Maynard. R. M. Bethke, chairman of the nominating committee, John R. Murlin, editor of The Journal of Nutrition and E. M. Nelson, chairman of the committee on vitamin nomenclature were called on for reports in connection with their activities. Formal actions of the Council are reported in the minutes of the general business session.

SCIENTIFIC SESSIONS

President Mary Swartz Rose presided at the general scientific sessions and business meeting. The scientific program was initiated at 9.40 A.M. March 30th, and proceeded on schedule time. All the papers listed on the program were given.

The six discussion groups attracted and held the interest of large audiences. The success of these conferences in satisfying the interest of the membership and friends in current investigation has been most gratifying. The group leaders have been most painstaking and skillful in planning for and promoting well-directed discussions by highly qualified speakers. It is evident that this phase of the program is one of the most attractive features of the annual meeting.

BUSINESS SESSION

The business meeting convened at 11.45 A.M. and was called to order by President Mary Swartz Rose. The reading of the minutes of the preceding meeting was dispensed with since they had been published in The Journal of Nutrition and could be read by all.

In accordance with the by-laws members in good standing who have reached the age of 65 years become emeritus members. It was announced that Thomas B. Cooley and Percy R. Howe have attained this rank of service.

The report of the treasurer was read by George R. Cowgill. The president appointed E. M. Nelson and H. E. Himwich to serve as auditors. They examined the treasurer's books and declared them correct. It was moved by Arthur Knudson and seconded by A. H. Smith that the report of the treasurer be accepted. The motion carried.

The council recommended to the members that the annual dues for 1938-1939 be \$1.00. It was so moved by Grace MacLeod and seconded by A. H. Smith. The motion carried.

In order to take care of increasing demands of the annual meeting the council recommended that a registration fee of 50 cents hereafter be charged to defray the expenses of the meeting; the surplus of previous years may be used for this purpose if a deficit occurs. It was so moved by I. McQuarrie and seconded by A. H. Smith. The motion carried.

The council recommended that the following candidates be elected to membership in the American Institute of Nutrition:

Herbert G. Barott
Carl A. Baumann
Ernestine Becker
Hugh D. Branion

William J. Dann
Henry J. Gerstenberger
Tom S. Hamilton
Margaret W. Johnston

Thomas H. Jukes
Paul H. Phillips
Tom D. Spies

A. R. Rose moved and Arthur Knudson seconded that the secretary cast a unanimous vote for the election to membership of the candidates approved by the council. The motion carried and the secretary declared the candidates duly elected members of the American Institute of Nutrition.

A summary of the questionnaire that was prepared and sent to the members to ascertain their wishes on the type of program desired and the place and time of meeting was presented by R. M. Bethke for the committee which included George R. Cowgill and Icie G. Macy. The answers indicated that the large majority of the members desired that the meetings of the American Institute of Nutrition be held in connection with the meetings of the Federation of American Societies for Experimental Biology and that the Institute remain independent for the time being to allow for further development of policies and experimentation with the program. Moreover, the answers indicated that on the whole the members were satisfied with the type of programs that have been suggested by the members and arranged by the council. It was moved by W. M. Sperry and seconded by E. M. Nelson that the 1939 meeting of the American Institute of Nutrition be held in connection with the Federation. The motion carried.

President Rose announced that the committee composed of R. M. Bethke, L. J. Roberts, W. C. Rose, I. G. Macy and herself for setting up standards for nutritionists in public health departments had fulfilled its duties. The report of the committee was approved by the council and forwarded to Martha M. Eliot of the Children's Bureau. It was also announced that the report is on file and copies may be obtained from the secretary by any of the members who desire to see it.

The activities of the committee on vitamin nomenclature were reported by the chairman, E. M. Nelson, for his collaborators, E. V. McCollum and H. C. Sherman. He announced that the Foods Committee and the Council on Pharmacy of the American Medical Association had expressed its willingness to give up the use of cevitic acid and accepted the use of ascorbic acid. The following report was given:

'Vitamin F'

The term 'Vitamin F' has been used in various ways in the past but recently has come into wide-spread use in promoting the sale of linseed oil and products alleged to contain so-called 'essential fatty acids.' A group of biochemists interested in fat metabolism gave consideration to this matter during our last

meeting. They forwarded to this committee their recommendation that the term 'Vitamin F' should not be used in referring to linoleic or linolenic acids, or so-called 'essential fatty acids.' Your committee is in accord with these views. It is recommended that the term 'Vitamin F' should not be used in referring to linoleic or linolenic acids or any fatty acids or mixtures of fatty acids.

It was moved by E. M. Nelson and seconded by George R. Cowgill that the term 'Vitamin F' should not be used in referring to linoleic or linolenic acids or any fatty acids or mixtures of fatty acids. The motion carried.

Riboflavin

At the last meeting your committee recommended that the term 'riboflavin' be used to designate the compound identified as '6,7 dimethyl-9 (d ribityl) isoaloxazine,' and that the terms 'vitamin B₂' and 'vitamin G' be no longer used. This recommendation was approved by the Society but final adoption was withheld until the opinions of other groups had been ascertained. No definite objections have been received to this proposal and it is therefore recommended that the term 'riboflavin' be formally adopted.

It was moved by E. M. Nelson and seconded by A. H. Smith that the term 'riboflavin' be formally adopted. The motion carried.

Vitamin A

Your committee has agreed upon a plan for choosing a name for Vitamin A to distinguish it from carotenoids of plant origin having Vitamin A activity. Investigators in this country and abroad will be consulted before a definite recommendation is made to the Society.

Editor John R. Murlin reported on the present status of The Journal of Nutrition. He announced the plan for the publication of a general index to The Journal of Nutrition following the completion of the fifteenth volume, the total cost of which would be about \$450. It is estimated that the subscription to members will amount to about 60 to 75 cents. The publisher has requested that the American Institute of Nutrition guarantee one-half of the total cost or \$225. It was moved by Doctor Murlin that the Institute go on record as approving the guaranty since there was little likelihood that it would be necessary to draw upon the Institute as the present subscription to the Journal would most probably take care of the cost.

After careful consideration the motion was amended by R. Adams Dutcher and seconded by V. C. Myers to leave the manner of meeting the deficit, if any, to the Council with power. Thus amended the motion carried.

Editor Murlin discussed the present status of the Journal in relation to the number of manuscripts sent in to those accepted and the economies being exercised by the new management of The Wistar Institute. The number of pages in a volume is being more rigidly limited to near 600 (the agreed-upon number) by the publishers. Numerous excellent manuscripts must now be rejected because of lack of publication space. In order to meet the present publication needs two possible solutions were considered, namely, reducing the length of the papers accepted or increasing the number of volumes of the Journal per year. Henry C. Sherman and others made a plea for more concise style in the papers that are published. The council recommended that The Journal of Nutrition be increased from two to three volumes per year when the editorial board and publisher think feasible, provided three-fourths of the members approve. It was moved by E. F. DuBois and passed by the Society that the recommendation of the council be accepted.

The council recommended the acceptance of a proposed award of \$1000.00 yearly to the American Institute of Nutrition by Mead Johnson and Company for a term of 5 years for meritorious research work on the 'B-complex.' It was moved by R. M. Bethke and seconded by L. C. Norris that the award be accepted and that a committee of five members be appointed by the chair to consider the details of the award. The motion carried. (President McCollum later appointed the following members to serve on the award committee: George R. Cowgill, chairman; L. A. Maynard, A. G. Hogan, I. G. Macy and C. A. Elvehjem.)

In accordance with the constitution President Rose named the following nominating committee for 1939: Victor C. Myers, chairman; I. McQuarrie, A. G. Hogan, Martha M. Eliot and R. C. Lewis.

President Rose asked the tellers, N. B. Guerrant, chairman, Helen A. Hunscher and H. H. Williams, to report on the count of the ballots for the election of officers for 1939. The results were as follows: President, E. V. McCollum; vice-president, T. M. Carpenter; treasurer, George R. Cowgill; secretary, L. A. Maynard; and councillor, Helen S. Mitchell. No new members of the editorial board were elected because no associate member had served the full term of 4 years.

Paul E. Howe moved that the secretary be given a rising vote of thanks for her 'excellent services' during the past 4 years. The Society so responded with enthusiastic applause.¹

It was moved by Henry C. Sherman and seconded by John R. Murlin that the secretary be instructed to extend heartiest thanks to the local committee and the management of the Southern Hotel for the splendid arrangements provided for the meeting and for the gracious services and courtesies extended to the members of the American Institute of Nutrition and their guests, all of which made the meeting so successful and enjoyable.

LUNCHEON

The noon luncheon in the Banquet Hall of the Southern Hotel was attended by 251 members and guests.

DINNER

A subscription dinner in honor of Prof. Russell H. Chittenden was held in the ball room of the Southern Hotel and was attended by 301 members and their friends. Among the honored guests were William H. Howell, William T. Porter and the presidents of each of the constituent organizations in the Federation of American Societies for Experimental Biology. Pres. Mary Swartz Rose traced in a delightful and entertaining manner the development and activities of the American Institute of Nutrition over its 5 years of existence. John R. Murlin, editor of *The Journal of Nutrition* served as toastmaster and introduced the honored guest with cogent remarks concerning his activities in the development of

¹ Editorial license.

physiological chemistry and nutrition in this country. Prof. Russell H. Chittenden spoke on "Some Changing Viewpoints in Nutrition."

EDITORIAL BOARD MEETING

The editorial board met at the call of the editor at the Southern Hotel, 6.30 P.M., April 1, 1938. The following members were present: George R. Cowgill, Howard B. Lewis, Arthur H. Smith, Grace MacLeod, Elmer M. Nelson, John B. Brown, William H. Chambers and Albert G. Hogan. The secretary, Icie G. Macy was in attendance to record the minutes of the meeting.

The editor reported on various matters concerning the relations of the journal to The Wistar Institute as publisher. These were, principally, the type of paper used in the journal, a new advertising policy of the Wistar, business arrangements for the general index to be published at the end of the current volume, and the possibility of increasing the number of papers published including expansion to three volumes a year. The board agreed to try for a year requiring authors to condense their material to an average length of not more than ten pages so as to accommodate ten articles in each number. This will be put into effect for all articles accepted after this date (April 1, 1938).

A discussion of policies and criteria for judging manuscripts followed. The associate editors exchanged ideas with the editor and the following criteria for judging manuscripts evolved:

1. Reasons for doing the work and whether the final results have scientific merit.
2. Evidence presented, i.e., adequacy of data.
3. Form and organization.
 - a. Use made of illustrative material.
4. Condensation of essential facts.

The 5-year term of John R. Murlin as editor being due to expire on January 1, 1939, the board was asked to consider the question and make some provision for filling the office. He

appointed Icie G. Macy, Secretary of the American Institute of Nutrition, to take the chair and conduct the election and he withdrew from the meeting. The members of the editorial board expressed their deep appreciation for the efficient and outstanding work of Doctor Murlin as editor in building up the Journal. Furthermore, they expressed a genuine desire to have him continue his office as long as it was not too great a burden for him. It was moved by Grace MacLeod and seconded by E. M. Nelson that John R. Murlin be re-elected for a 5-year period as editor of The Journal of Nutrition from January, 1939. The motion carried unanimously. Doctor Murlin accepted the re-appointment on the understanding that he would resign the moment he felt he could not carry on the editorial work efficiently.

There was consideration of the need for an assistant editor to relieve the editor of some of his routine responsibilities. It was moved by Howard B. Lewis and seconded by E. M. Nelson that Doctor Murlin be allowed to select an assistant editor for the coming year. The motion carried.

Respectfully submitted,

ICIE G. MACY, *Secretary*

American Institute of Nutrition

ABSTRACTS OF PAPERS

The measurement of the efficiency of diets. New apparatus and procedures.

E. B. Forbes, R. W. Swift and Alex Black (by invitation), Institute of Animal Nutrition, Pennsylvania State College.

New equipment was devised and two experimental procedures were demonstrated, with growing albino rats as subjects, for comparing and measuring nutritive values of human diets.

The equipment was a water-sealed respiration chamber permitting normal physical activity of the subjects.

One procedure was a growth experiment, with carbon and nitrogen balances, at intervals, for observing the effects of the progressively increasing severity of the nutritive conditions imposed by a constant food intake, without allowance for the growth of the subjects.

This procedure yielded results in terms of gross efficiency, covering requirements for maintenance and growth together.

The second procedure consisted of nutritive measurements, also by means of balances of carbon and nitrogen, but in terms of net energy and protein.

Balances were determined during fast, at the maintenance level, and at a higher plane, and values were determined for metabolizable energy, heat increment, and net energy—separately for maintenance and growth.

Six human diets, differing in significant ways, were compounded to contain the same gross energy and protein contents. Two of these were compared by the constant food intake method, and all six by the net energy procedure.

The fat composition of milk fed rats. Herbert E. Longenecker (introduced by R. A. Dutcher), Department of Industrial Chemistry, University of Liverpool, England.

This constitutes a detailed examination of the neutral fat fatty acids from rats which were fed milk only for 11 weeks after weaning. An electrically heated and packed fractional distillation apparatus¹ of high efficiency was used for the separation of fatty acid ester mixtures. The analyses indicated 1) that fatty acids lower than C_{10} were not stored in detectable amounts and only 1.4 mols.% of the mixed rat fatty acids consisted of C_{10} and C_{11} acids whereas similar analyses of milk fat² show about 23 mols.% of C_4 , C_6 , C_8 , C_{10} and C_{12} acids, 2) a somewhat higher myristic acid content than is usual for rat fat which may be related to the quantity of this acid in milk fat as a direct example of the deposition of a food fatty acid, 3) the presence in this rat fat of tetradecenoic acid, not previously reported, which may be either a normally expected component or one contributed by the milk fat, and 4) that on a relatively low fat diet, the rat apparently constructs a body fat of nearly constant composition containing oleic (45 to 50 mols.%) and palmitic (25 mols.%) acids with smaller amounts (3 to 6 mols.%) of myristic, stearic, hexadecenoic and octadecadienoic acids, and traces of C_3 saturated and unsaturated acids.

¹ Longenecker, H. E., J. Soc. Chem. Ind., vol. 56, p. 199T (1937).

² Hilditch, T. P., and H. E. Longenecker, J. Biol. Chem., vol. 122, p. 497 (1938).

Nicotinic acid in the prevention of blacktongue of dogs. W. H. Sebrell, R. H. Onstott (by invitation), H. F. Fraser (by invitation) and F. S. Daft (by invitation), National Institute of Health.

Five dogs were given a blacktongue producing diet with the addition of nicotinic acid in amounts varying from 1 to 50 mg. biweekly. No blacktongue occurred during the 6-month experimental period, except in the animal receiving 1 mg. One animal died, possibly of riboflavin deficiency. A weekly supplement of 0.5 mg. of riboflavin was then given to each dog.

Five dogs developed blacktongue on the same diet without nicotinic acid. These animals were treated with varying amounts of nicotinic acid until the acute symptoms subsided. They were then maintained in good condition to the end of the experiment on 10 mg. biweekly.

Seven dogs developed blacktongue on the same diet without nicotinic acid and repeated attacks of blacktongue were successfully treated with varying doses of nicotinic acid, which was discontinued when the acute symptoms of each attack subsided.

Synthetic and commercial nicotinic acid were used.

The relation of the vitamin B complex to fat metabolism. E. G. Gavin (by invitation) and E. W. McHenry, School of Hygiene, University of Toronto.

Previous work by Whipple and Church and by us has shown that thiamin causes a formation of fat from carbohydrates in rats; this synthesized fat accumulates in the liver when the diet lacks choline and in the depots if the diet contains ample choline. Supplee and others have reported that rice polish concentrate, used as a source of vitamin B₆, augments the effects of thiamin and flavin in increasing the weight of young rats. The present work indicates that this increase in weight is partially due to a marked effect of the concentrate on body fat, an effect which is not due to the amount of choline in the concentrate. Choline, flavin and thiamin either separately or in several combinations do not give this effect. It is suggested that at least part of the supplementary effect of several members of the vitamin B complex on increases in body weight is due to their relation to fat metabolism. Thiamin causes an accumulation of dietary or synthesized fat in the liver. Vitamin B₆, or another component of rice polish concentrate, promotes a storage of fat in the depots. The effect of rice polish concentrate is augmented by flavin.

Variations in the reproductive behavior of different species of mammals restricted to vitamin E-deficient rations. Byron H. Thomas, C. Y. Cannon (by invitation), S. H. McNutt (by invitation) and Gravers Underbjerg (by invitation), Animal Chemistry and Nutrition, Iowa State College.

Whether rations deficient in vitamin E will interfere with the normal course of reproduction in farm livestock as they do when fed to rats has not been determined heretofore.

We have restricted goats, sheep and rabbits during one or more generations to specially prepared feed mixtures which repeatedly have produced resorption gestations in female rats. The vitamin E in these mixtures of ground grains and finely chopped alfalfa hay was destroyed or inactivated by treatment with an ether solution of ferric chloride and subsequently aging them until after they had acquired a distinctly rancid odor. The mixtures were fortified at feeding time

with a vitamin E-free supplemental mixture compounded primarily to protect against possible other vitamin deficiencies.

Male and female goats were able to reproduce through several generations apparently unhampered when restricted to vitamin E-deficient rations which regularly produced resorption gestations when fed to female rats. Results of a preliminary investigation of 2 years duration involving male and female rabbits agree in part with those yielded by the goats. A number of young lambs have been limited to similar feed mixtures for 1 and 2 years. All have been bred for spring lambs. Some already are exhibiting unmistakable signs of advanced pregnancy. The studies involving rabbits and sheep are being continued.

The foregoing observations appear to have important scientific and practical implications.

Effects of ascorbic acid upon resistance to bacterial toxins. C. G. King, A. Sigal (by invitation) and R. R. Musulin (by invitation), Chemistry Department, University of Pittsburgh.

The protection afforded by different levels of vitamin C intake has been studied in relation to tissue injury and physiologic impairment caused by injections of bacterial toxins. Guinea pigs were fed a modified Sherman diet, supplemented by 0.5 to 5.0 mg. of synthetic ascorbic acid per day. An intake of 0.5 mg. of vitamin per day permits nearly normal growth and affords protection from gross signs of scurvy, but animals receiving 5.0 mg. of vitamin per day were less sensitive to injury by bacterial toxins than were animals receiving only 0.5 mg. per day. The teeth provide a particularly good index to the correlation between nutritional level and tissue injury. Studies of blood cellular constituents, arterial lesions, sugar tolerance, length of survival, and changes in body weight provide less sensitive but confirmatory evidence of the benefit that may be derived from a generous level of ascorbic acid intake in comparison with a level that is barely protective against scurvy. The differences in degree of injury that can be correlated with differences in nutritional level have been found to vary greatly with different toxins. Diphtheria toxin has proved to be the most satisfactory of those studied.

Distribution of vitamin K in biologic material and its probable physiologic significance. Hugh R. Butt, Arnold E. Osterberg (introduced by R. M. Wilder), Mayo Clinic, Rochester, Minn.

A few years ago, Dam discovered a sterol substance in pig liver fat (vitamin K), which would prevent the occurrence of a hemorrhagic diathesis in chicks fed a deficient diet. It was postulated that the absence of this sterol might be indirectly related to the decreased level of prothrombin observed in the circulating blood of these bleeding animals. In individuals with obstructive jaundice there is likewise an apparent decreased quantity of prothrombin in the circulating blood, and in these individuals a hemorrhagic diathesis is not infrequent. The possibility of a deficiency or inadequate absorption of Dam's sterol substance occurring in individuals with obstructive jaundice is considered and the method for preparing this sterol substance from fish meal is described. Its effect upon the prothrombin level of the blood of individuals with obstructive jaundice when administered alone or with bile is discussed, and its probable distribution in biologic material is mentioned.

Some pathological and bacteriological effects of chronic vitamin A deficiency.

Aline Underhill Orten (by invitation), Caspar G. Burn (by invitation) and Arthur H. Smith, Departments of Physiological Chemistry and Pathology, Yale University.

A mild chronic or incipient vitamin A deficiency was produced in young rats and the condition maintained for periods up to 1 year. The quantities of vitamin A (I. U.) required to bring about this state have been determined and their relation to age, weight and sex studied. The animals were examined daily and at appropriate intervals groups were sacrificed in order to study the progressive development of pathological and bacteriological changes. The findings have been compared with those from normal control rats for the same age and with those from young animals showing the classical symptoms of advanced vitamin A deficiency.

Slightly subnormal growth, respiratory infections and a relatively high mortality (54%) occurred. The incisor teeth showed progressively loss of pigmentation, development of an opacity, distortion and eventual exfoliation of the erupted portion. Large, non-sensitive growths developed in the maxillae. Histological changes occurred in the incisor teeth similar to those found in the typical, acute A-deficient animals and, in addition, tumor growths (odontomas) and supernumerary teeth had developed. Bacteriological changes were also observed in the chronic A-deficient animals. A shift from the saprophytic flora normally found on the various lining epithelia to potentially pathogenic forms, and eventual invasion of the viscera by these organisms occurred.

The retardation of senescence. C. M. McCay, L. A. Maynard, Gladys Sperling (by invitation), Animal Nutrition Laboratory, Cornell University.

New experiments have been completed to determine the effect of different periods of retardation of growth upon the development of the characteristics of old age and upon the length of the total life span. At the time of weaning white rats were divided into five groups. The first group which represented the control was allowed to grow to maturity at a normal rate. The remainder of the groups was retarded by calorie restriction for periods of 300, 500, 700 and 1000 days, respectively, and then allowed to complete their growth.

An intensive study has been made of these individuals to determine such factors as the growth of the bones, the degree of calcification of the arteries and the cause of death. In the present report attention will be centered upon the retardation of senescence that resulted from slowing the growth rate.

Although the 'normal' growth group originally contained a third of all the animals, the last of this group died at the age of 964 days, leaving animals alive in all the retarded groups. Photographs were used to show the contrast between the 'old' members of the normal growth group and the retarded ones. At the age of 1068 days 12% of the retarded animals were still alive in spite of losing approximately half of them from accidents in the first year of the experiment.

These data indicate that the retardation of growth affords a method of retarding senescence and extending the span of life far beyond the normal. The method provides an effective technic for attacking the problems of aging within the body of experimental animals.

Total energy metabolism of rats at various ages as affected by protein and exercise. A. Black (by invitation) and J. R. Murlin, Department of Vital Economics, University of Rochester.

The total energy metabolism of ninety-six albino rats, comprising six groups of equal number, was studied intermittently for 8½ months.

The protein fed these animals was supplied by whole milk powder and dried whole egg. There were three groups of animals on each protein treatment, two of which received a ration containing 20% protein and one 8% protein. One of the groups given high protein from milk and one from eggs were exercised a definite amount daily. The three groups of animals on a particular protein treatment were fed according to the paired-feeding method as modified to match three animals instead of two. The total energy consumption of all six groups was essentially the same.

The animals, which received their protein from eggs, made greater gains in live weight than was noted with milk. The greatest difference occurred on the 8% protein level and the least difference when exercise was imposed.

The heat production, as measured with a new automatic calorimeter, of the three groups receiving milk was the same on a unit basis showing a decrease with age until the last month when an increase occurred. The exercised group on eggs produced more heat than the two groups which were not exercised. The delayed increased heat production occurred earlier in these groups than in the milk fed animals.

Basal metabolism tended to be slightly higher in the exercised animals.

Effects of prolonged use of extremely low fat diet on adult human subject. W. R. Brown (by invitation), A. E. Hansen (by invitation), G. O. Burr and I. McQuarrie, Departments of Pediatrics and Botany, University of Minnesota.

As a sequel to studies, which suggested an etiological relationship between infantile eczema and a disturbance in the unsaturated fatty acid metabolism, the present investigation was undertaken to determine whether or not the human subject requires preformed fat in the diet, as the young rat does. In the absence of a justifiable opportunity to make such tests on the growing infant, a normal human adult was observed over a period of 6 months while subsisting on a diet practically devoid of fat. Twelve young rats placed on this experimental diet developed typical signs of the fat deficiency disease described by Burr and Burr.

While careful weekly examinations of the experimental subject revealed no clinical evidence of disease as a result of the diet, he manifested certain changes in metabolism toward the end of this period, which are characteristic of the fat-deficiency syndrome as it occurs in rats. The R.Q. following a meal of the diet rose to 1.14 at the end of the period, whereas the maximum at the beginning was 0.99. The iodine number of the serum fatty acids fell from an average of 122 to 92. Direct determinations of the linolic and arachidonic acids of the serum showed them both to decrease approximately 30% during the experimental period. From these findings it is concluded that the human subject, like the rat, requires preformed unsaturated fatty acids in the diet.

Influence of Ca: P ratio of diet on ratio in serum during development and cure of low phosphorus rickets. Henry J. Gerstenberger (introduced by Victor C. Myers), Laboratory of Babies and Childrens Hospital and Department of Pediatrics, School of Medicine, Western Reserve University.

This report presents part of a comprehensive study of rickets in monkeys (*Macacus rhesus*). Work previously completed has shown that a low phosphorus type of rickets develops regularly and rather easily in monkeys in a sunlight free environment, just as in full-term human infants, even though the diet fed the monkeys has a low calcium, high phosphorus ratio (0.49).

The present report deals with the response of monkeys fed rickets-producing diets, a) with a Ca: P ratio of 0.49, the original diet, and b) with a ratio higher in calcium, more nearly approaching that of cow's milk, 1.3, to treatment with ultraviolet light.

The entire group included twenty-seven monkeys; fourteen were exposed six times weekly to $\frac{1}{2}$ or 1 M.P.E. doses of ultraviolet light and the remainder served as controls.

Of the fourteen monkeys exposed, six had been fed since admission the diet with Ca: P ratio 1.3 and eight the diet with Ca: P ratio 0.49. Progress and kind of healing were checked by determinations of calcium and phosphorus in the blood serum and by the degree of healing of the bones as shown by roentgenograms.

It was found that, under the conditions chosen, the higher Ca: P ratio of the diet used was of greater influence in preventing the drop in the calcium level of the blood serum than was the higher dosage of ultraviolet light.

The role of manganese in bone development. L. C. Norris, W. D. Gallup (by invitation) and C. D. Caskey (by invitation), Department of Poultry Husbandry, Cornell University.

In studies on the role of manganese in nutrition it has been found that manganese is essential for normal bone development in the chick. The tibiae of day-old chicks from hens fed a low-manganese diet (13 p.p.m.) were found to be 12.5 to 13.1% shorter than those of chicks from hens fed a high-manganese diet (200 p.p.m.) and the metatarsi were found to be 14.2 to 15.1% shorter.

The long bones of the legs and wings of chicks fed a low-manganese diet (5.5 p.p.m.) were found at 3 weeks of age to be shorter than those of comparable chicks fed a high-manganese diet (100 p.p.m.). By comparing the femurs of paired chicks it was found that the odds against the shortening of the femurs of the low-manganese chicks being due to chance were 713 to 1 at 3 weeks of age and 3332 to 1 at 4 weeks of age.

This phenomenon is not caused by a disturbance in gross body composition, as the percentage of water, fat and ash of the bodies of paired chicks was essentially the same. The gross composition of the femurs of chicks 3 weeks of age was also approximately the same. However, the manganese content of the leg bones of the low-manganese chicks was less than that of the high-manganese chicks.

ABSTRACTS OF PAPERS READ BY TITLE

On the fatty acids essential in nutrition. G. O. Burr, J. P. Kass (by invitation), J. B. Brown and Jerome Frankel (by invitation), Departments of Botany, University of Minnesota, and Physiological Chemistry, Ohio State University.

Oils can be biologically assayed for their content of essential fatty acids. Although such an assay puts them in an order of curative power corresponding in general with their unsaturation and linoleic acid content as reported in the literature, there are outstanding exceptions. Corn oil is superior to linseed oil. Purified oleic acid from several sources continues to give negative results.

Linoleic acid prepared by debromination of the tetrabromides has a curative value similar to that in the original oil. On the other hand, the so-called β -acid which gives no insoluble tetrabromide in petroleum ether seems to be much less efficient than the α -acid.

Arachidonic acid is also of high curative value. The isomeric fatty acids and their nutritive values are discussed.

Growth of suckling rats in relation to the diet of the mother. Gerald J. Cox, Sara F. Dixon (by invitation), Margaret C. Matuschak (by invitation) and W. Edgar Walker (by invitation), Nutrition Fellowship, Mellon Institute.

The individual weights of 508 suckling rats in seven groups were determined daily from birth to weaning at 21 days. The mean weights at weaning varied from 31.9 gm. on a cornmeal-milk powder diet to 50.3 on a meat-calcium carbonate ration.

Conclusions were as follows: 1) The rate of growth of suckling rats is a function of the diet of the mother. 2) The coefficient of variation from all rats was about 10% at birth, a maximum of about 16% at 10 days and improved to 12% at weaning. 3) The better the diet of the mother, the lower the coefficient of variation. 4) Growth rate declines continuously after the fourth day. 5) There is no evidence of abrupt change of growth rate. 6) Weaning weight is positively correlated with birth weight.

Groups A and B of six rats each were suckled successively by three mothers for 8-hour periods so that group A was always suckled by a mother rested for 8 hours and group B suckled the next period after group A. The Anderson and Smith diet was used. Eighteen group A rats averaged 67.3 gm. at 21 days; eighteen group B rats averaged 47.7.

Comparative mineral balance studies with children receiving two levels of pasteurized and evaporated milk in high and low cereal diets. Amy L. Daniels, Iowa Child Welfare Research Station, University of Iowa.

The substitution of superheated milk, more particularly evaporated, for raw or pasteurized in the human dietary, has led to a study of its nutritive value by comparing the calcium, phosphorus and nitrogen retentions and fecal excretions of nineteen preschool children receiving during two successive metabolism periods the same diets with comparable amounts of pasteurized and evaporated milk. The vitamin D intake was the same in all cases; and the vitamin C was adequate. High and low cereal diets were tested with two levels of milk ingestion.

Some clinical studies on vitamin C deficiency. William Freeman and W. Everett Glass (introduced by M. O. Lee), Worcester State Hospital, Worcester, Mass.

A sample of a mental hospital population showed that 53% of the psychotic patients and 28% of the healthy, normal employees had a subnormal fasting blood vitamin C level. None of the subjects showed any signs of scurvy.

No correlation was found between the ascorbic acid blood level and the age, sex, psychotic state, length of hospitalization, nutritional index, rectal temperature, systolic blood pressure, pulse pressure and blood chemical and morphological elements. Neither were there any correlations found between the blood vitamin C level and the incidence of bleeding gums, gingivitis, and the number of capillary skin hemorrhages.

Central autolysis of the adrenal glands at autopsy was found to be correlated with a subnormal antemortem ascorbic acid level of the blood.

The ascorbic acid content of various organs at autopsy showed a wide range of variation and was not parallel with the post mortem blood level. When a deficient level was obtained, the ascorbic acid content of the adrenal glands was the lowest of any organ, and when an adequate level obtained, the adrenal glands had the highest concentration.

Bringing the blood ascorbic acid from a subnormal point to one well above the minimum normal level required varying amounts, between patients, of daily intravenous administration of vitamin C. To maintain the blood at a constant, normal level also required varying amounts, between patients, of daily intravenous administration.

The influence of calcium and phosphorus on iron assimilation. S. W. Kletzien, State Institute for the Study of Malignant Disease, Buffalo, N. Y.

In previous communications it has been shown that various inorganic salts of calcium depress the normal assimilation of iron in the young growing rat, and that calcium carbonate in the amounts fed interfered with the maternal and foetal storage of iron. It is now found that the citrate, tartrate, and acetate of calcium do likewise, and that the malate and oxalate do so to a lesser degree.

As regards phosphorus, the surmise exists that it in some way interferes with maximum iron assimilation. In the light of our findings this is not substantiated. Animals (rats) receiving phosphoric acid as such assimilated more iron than control animals receiving an equivalent amount of phosphorus as tri-calcium phosphate. Lower iron values are obtained only with the simultaneous introduction of calcium. In regard to calcium/phosphorus ratios, evidence is accumulating which supports the belief that a low Ca/P ratio improves iron assimilation appreciably.

The vitamin B₁ retentions of infants. Elizabeth Knott (by invitation) and Frederic W. Schlutz, Department of Pediatrics, The University of Chicago.

Retentions of vitamin B₁ have been investigated for four infants during a series of controlled metabolism periods. Each infant has been studied for at least ten periods of 5 days duration each. Urine, feces, and food have been analyzed for their vitamin B₁ content by means of carefully controlled biological assay.

By altering the level of vitamin B₁ ingestion, the optimum intake has been determined which will give maximum retention of the vitamin.

In comparing results with those previously reported for preschool children, it is noted that a higher requirement of vitamin B₁ is needed per unit of body weight for infants than for the older children. With the infants, the vitamin is almost completely absorbed as contrasted to the considerable quantities excreted in the feces of older children. Urinary excretion of vitamin B₁, in both infants and children, increases with increased intakes, but at a slower rate than the intake if the child has been receiving suboptimal amounts. If optimum or above optimum amounts are ingested, urinary excretion increases until a balance is established which seems to suit the child's required needs.

Effect of topical application of cod liver oil on wound healing and on liver vitamin A in normal and chronically A-deficient rats. Elaine P. Ralli and Harold Brandaleone (by invitation), Laboratories of Department of Medicine, New York University College of Medicine.

Groups of rats rendered chronically deficient in vitamin A were wounded under aseptic conditions at the time the symptoms of vitamin A deficiency appeared. In one group of animals the wounds were treated with weighed amounts of a standard cod liver oil; in the other group of rats no cod liver oil was applied to the wound. Groups of normal rats were similarly wounded. The rate of healing in each group was determined by measuring the wounds daily and by noting the time at which healing occurred. In all groups at the time the animals were placed on the A-deficient diet a certain number of animals were sacrificed and the vitamin A content of the livers determined. This was done again at the time the symptoms of vitamin A deficiency appeared, and at the end of the experiment the vitamin A content of the livers was determined. It was found that when the wounds in the A-deficient animals were treated with cod liver oil the rate of healing was twice as fast as in the untreated wound, but the vitamin A content of the liver in the group whose wounds were treated with cod liver oil was not higher. It is suggested that cod liver oil has a specific effect on wound healing not necessarily related to vitamin A.

Weights of diabetic organs in relation to a possible pituitary influence. Howard F. Root, George F. Baker Clinic, New England Deaconess Hospital, Boston, Mass.

Since the demonstration that it is possible to produce permanent experimental diabetes in the dog by injection of an extract of the anterior pituitary lobe of oxen, it is important to investigate the possibility that some diabetogenic hormone is a factor in the causation of many if not all cases of diabetes. The high incidence of diabetes in acromegaly clearly indicates that a diabetogenic factor is present in these cases in association with whatever factor it is which produces the great increase in size not only of the skeleton but of the internal organs. If such a diabetogenic factor is an important agent in producing clinical diabetes one might expect some correlation between the size of the pancreas and the size of other organs. In acromegaly the pancreas as well as other tissues is often enlarged and by contrast, in Simmon's disease the pancreas as well as other

organs shows atrophy. In analyzing 200 diabetic autopsies, studies of tissue weights of those cases in which the pancreas was notably small was made in comparison with cases in which the pancreas was notably large. A study of the relationships in comparison with acromegalic diabetes is presented.

Cost and nutritive value of American diets. Hazel K. Stiebelling, Bureau of Home Economics, U. S. Department of Agriculture.

As part of a nation wide study of family consumption conducted by federal agencies, dietary information from about 25,000 non-relief families has been analyzed after classification by region, degree of urbanization, color of family, level of expenditure for food, and to some extent also by season, occupation and the composition of the family.

A comparison of the nutritive value of the food received in the kitchen during 1-week periods with specifications for 'good diets' essentially the same as those proposed by the League of Nation's Technical Commission on Nutrition indicates that with present day food habits and 1936 prices families require more than \$90 per person per year to buy 'good diets,' some require as much as \$250. Expenditures for food by the interquartile group fell between approximately \$100 and \$160 per person per year. The dietary deficiency most frequently encountered was calcium. The highest proportion of 'good diets' was found on the Pacific coast where the consumption of milk, fruits and the succulent vegetables was highest for any section of the country, and the lowest in the southeast where sugars and fats tend to displace these protective foods. As a rule farm family diets were found to be better than those of city families; white family diets better than Negro, and diets of families including young children better than those composed only of adults.

Cellular changes in the anterior hypophyses of vitamin A deficient rats. T. S. Sutton and B. J. Brief (by invitation), Dairy Industries, Ohio Agriculture Experiment Station and Department of Anatomy, Ohio State University.

Differential cell counts were made on anterior hypophyses from normal, vitamin A deficient, and castrate rats, in an attempt to determine the 'modus operandi' of vitamin A deficiency in causing sterility. It was found that a deficiency of vitamin A caused an increase in the percentage of beta (basophilic) cells, together with an increase in the number of beta cells containing a macula. This increase in beta cells was significant in vitamin A deficient animals of both sexes. However, when compared to normals, a greater relative increase in beta cells was noted in hypophyses from vitamin A deficient males than from vitamin A deficient females. The changes noted in vitamin A deficiency approach those found in true castrates. The greater relative change in vitamin A deficient males, when compared to similar vitamin A deficient females, may be accounted for on the basis of a more nearly complete destruction of the germinal epithelium of the male gonad.

This study presents further evidence that a vitamin A deficient diet causes direct damage to the gonad, the increase of beta cells representing a compensatory change in the hypophysis.

Toxemic pregnancy in the rat. Pearl P. Swanson and P. Mabel Nelson, Nutrition Laboratory, Food and Nutrition Department, Iowa State College.

If rats are fed a supposedly adequate diet containing dried canned pork muscle as its protein constituent, as many as 30% of the females may die in parturition, exhibiting many signs of toxemia. Symptoms develop suddenly; muscle tone disappears, body temperature falls, the rat trembles, respiration becomes rapid and labored, bloody urine is excreted. Severe convulsions with drooling from the nose and mouth follow. Autopsy shows an extremely yellow and friable liver with congestion, hemorrhage, or degeneration of the kidney. A reddish serous fluid surrounds the lungs. The pancreas is embedded in a jelly-like mucous. The blood is watery and does not clot. Histologic studies are being made, as well as chemical determinations of the glycogen and fat content of the liver.

Two grams of fresh liver as a daily dietary supplement protected ten rats through four successive pregnancies. Choline and lecithin, tested as possible potent factors in liver, aggravated the occurrence of the syndrome as did liver extract. However, lipocaic, isolated from pancreas by Dragstedt at a daily dosage of only 4 mg. was fully protective in forty littersings.

The fatty appearance of the liver suggests a deranged fat metabolism. If a pancreatic hormone is concerned with fat metabolism, apparently the diet fed does not provide precursors for the elaboration of sufficient lipocaic to meet the needs of pregnancy.

GROUP DISCUSSIONS

3 to 5 P.M., March 30th

1. *The use and place of the chick in nutritional studies.* R. M. Bethke, presiding.

The chick has a definite place as an experimental subject a) in the investigation of the nutrition of the species itself and b) for the study and assay of various nutritive factors. It has already proved valuable in the differentiation of the several antirachitic factors, in the assay of vitamin K (anti-hemorrhagic factor) and in blood regeneration studies. One method for the assay of antirachitic potency specifies the use of the chick. The study of embryos from the eggs of hens on a low vitamin E ration has revealed information concerning rapid cell proliferation. The chick has been used extensively in the study and differentiation of the members of the vitamin 'B and G complex.' Also in the study of the nutritive value of proteins it has been of importance. Our increased knowledge of the nutritive requirements of the species has permitted the formulation of more satisfactory synthetic rations.

Success in the use of the chick in the laboratory is determined by genetic factors, freedom from disease, selection as to age, sex, nutritional history of the hens, hours of light, temperature and humidity, physical properties of the feed, training it to eat and the method of distribution in experimental pens.

2. *The significance of dietary fat.* J. B. Brown, presiding.

Following some introductory remarks by the chairman, the subject of dietary fat as affecting digestion and absorption was discussed by J. P. Quigley. Comments and questions by members of the group were directed largely at the question of the possible effects of ingested fats on the absorption of other food constituents.

G. O. Burr then described the more recent findings on the essential nature of fat. He pointed out that no one fatty acid can be regarded as the essential one but rather that several serve; however, the responses to the administration of the individual acids vary in rapidity and degree of effectiveness.

The practical value of dietary fat in the treatment of disease was discussed by I. McQuarrie largely from the standpoint of the uses of low and high fat diets. Comments by other workers in the field showed the wide applicability of controlled diets and the wide field for further clinical study.

W. M. Sperry then presented the problem of optimum fat intake in man. He pointed out that both quality and quantity are concerned. The quality is probably of less practical importance than the quantity because of the complex nature of most natural fats and of the characteristics they have in common. Experimental work has shown that a high fat diet is less conducive to sustained muscular effort than a high sugar diet yet no exact standards of optimum intake are as yet available.

The discussion closed with some observations on the possible toxic effects of dietary fats. Approximately 110 members and guests attended the meeting of whom approximately fifteen joined in the discussion led by the listed speakers.

3. Members of the vitamin B complex. C. A. Elvehjem, presiding.

A mimeographed outline of the factors included in the vitamin B complex prepared by the chairman was passed out to all those present. W. H. Sebrell opened the program with a movie on the production and cure of blacktongue in dogs. This was followed by a report from Tom Spies on the successful use of nicotinic acid in the treatment of a large number of cases of pellagra. There was a great deal of interest in the colored photographs showing the dramatic responses obtained after the administration of nicotinic acid. After these papers there was considerable discussion on the subject of pellagra and nicotinic acid.

Paul György continued with a brief but complete survey on vitamin B₆. He especially emphasized the recent work on the isolation of vitamin B₆ in crystalline form. This paper also led to a number of questions on the relation of other factors such as fat and flavin to vitamin B₆ deficiency. W. J. Dann outlined the work which has been done on the filtrate factor, specifying that the filtrate factor referred to the chick antidermatitis factor. He pointed out that very few laboratories were working on the filtrate factor and that undoubtedly the so-called filtrate contains several rather than one factor. E. M. Nelson concluded by referring in a general way to the accepted nomenclature for the B factors and dealt specifically with the possibility of a more specific name for the chick antidermatitis factor. After his paper there was still considerable discussion on the entire subject. About 275 attended this group discussion.

4. Methods of judging nutritional status and requirements. Lydia J. Roberts, presiding.

Great interest was shown in the question "Does the Biophotometer Measure the Vitamin A Stores?" The problems involved in the validity of the test and possibility of a straight line relation of the readings to vitamin A stores were discussed. Controversy was raised concerning reproducibility of data, learning factor, standard units for recording means of converting findings in universal terms, standards for adults and children and the effect of supplementing the diet with vitamin A. The necessity for consideration of visual acuity, method of controlling light, standard position for most sensitive portion of the eye and calibrations of instrument were stressed. So many points of view were expressed that it is difficult to state conclusions except that the principle of the test is valuable in sorting out the individuals with subclinical deficiencies but for precise determination of degree of subnormal vitamin A nutrition standardization of method in terms of the physiology of the eye needs further investigation. Many people took part in the discussion which was led by workers from the U. S. Public Health Service, The University of Chicago and the Bureau of Home Economics, U. S. Department of Agriculture.

The topic "The Urinary Excretion of Ascorbic Acid as a Measure of the Body's Store and Requirement of This Vitamin" was introduced by H. M. Hauck. The method can be used to determine the amount needed to produce saturation of the body whereas random measurement does not indicate the adequacy of the diet. Large test doses administered after saturation of the body give reliable data on requirement. Twenty-five milligrams for 6 successive days followed by a test dose of 400 mg. were found inadequate for saturation, whereas,

75 mg. daily were adequate. Factors other than diet to be considered in using these methods are environmental temperature, effect of drugs, reaction of diet and non-specific reducing substances in the urine.

Estelle E. Hawley opened the question of "Reduced Blood Ascorbic Acid as a Measure of Vitamin C Stores." The level in the blood is a valid indication of the state of vitamin C stores. The range of data rather than absolute values must be considered. Concentrations below 0.5 mg. per 100 cc. indicates scurvy; 0.5 to 0.8 mg., low normal; 0.8 to 1.0 mg., adequate, and 1 or more, optimal vitamin C nutrition. Amounts of ascorbic acid in the diet were related to the levels found in the blood.

5. Mineral elements in nutrition. Henry C. Sherman, presiding.

E. V. McCollum stated that the older work indicated that Na, Cl, K, P, Ca, and Fe are essential. The modern era begins with Baumann's discovery of I in thyroid, and now Cu, Mn, Zn, Mg, and Co are added to the indispensable mineral elements. The question regarding essential nature of Br and B is not settled; Al and F seem unnecessary whereas the status of As, Se, Rb, Va and Ni is not settled. Considerable time was spent discussing the newer features of the deficiency of Mg, Mn, and Co.

A. H. Smith confined his discussion to the recent contributions which showed the influence of mineral nutrition on the blood. The present status of milk anemia and the significance of Fe and Cu was emphasized. The importance of normal gastro-intestinal function on the utilization of Fe as shown by hematopoiesis was emphasized. The effect of a deficiency of Mn, Mg and Co on the blood picture was discussed. The significance of mineral nutrition in maintaining a normal state in the blood was emphasized both from the point of view of scientific interest and of practical importance.

Genevieve Stearns pointed out the uncertainty in depending on mineral analyses of the skeleton in the literature. The proportion of water and of minerals are inversely proportional in the skeleton just as in the body as a whole. There are 1500 to 2500 gm. of Ca in the adult skeleton, the rate of growth influencing this. The essential proportionality between intake and retention of calcium by young children was emphasized as was the dependence upon vitamin D by children in several age groups. Need for Ca is greatest during lactation when 1.5 gm. per day with vitamin D is indicated. A minimum of 1 gm. per day with vitamin D is recommended for children. The enhanced retention of Ca with vitamin D is not to be taken as reducing the amount of Ca necessary in the diet.

E. J. Underwood, of New Zealand, discussed the minimum requirement of Co at length indicating that for calves 0.1 mg. per day was enough. In response to a question by A. H. Smith, Genevieve Stearns indicated a superiority of the water-soluble preparations of vitamin D. W. D. Armstrong also made comments.

6. *The relation of vitamins to clinical conditions.* Frederick F. Tisdall, presiding.

Vitamin A

C. D. May: Cornified epithelial cells in areas where such do not ordinarily occur means a vitamin A deficiency. A very severe deficiency over a long period of time is necessary to produce this cornification.

Recently many people have been attempting to measure light adaptation and using this as a means of measuring vitamin A deficiency. This test involves a moderate degree of intelligence in and a great deal of application by the subject. Other factors besides vitamin A have to do with light adaptation. There is a quantitative relation between the amount of vitamin A and the blue color produced in the Carr Price reaction. For the past 2 years we have been using the Evelyn photoelectric colorimeter for measuring the color produced in this reaction.

Vitamin C

K. Evelyn demonstrated the use of the photoelectric colorimeter devised by him in the measurement of ascorbic acid. By the use of this instrument, consisting essentially of suitable filters, a photo-electric cell and a galvanometer, the reduction of the indo-phenol reagent can be rapidly and accurately determined when an ascorbic acid solution is added to an excess of the dye. The effect of interfering substances upon the rate and amount of reduction was discussed.

W. H. Eddy pointed out that there was a good deal of interpretation to do in vitamin C work even after precision measurements had been made by an instrument such as Doctor Evelyn's. In his own work he pays no attention to single estimations on the ascorbic acid content of the blood, but administers 500 mg. of ascorbic acid by mouth and then by hourly measurements of the ascorbic acid content of urine and blood is able to obtain information concerning the state of nutrition with regard to vitamin C. He found that per unit of size California and Florida oranges were equally valuable in ascorbic acid. While the one had a higher ascorbic acid content per unit of juice, the other yielded more juice. During storage there is little loss of vitamin C in oranges, and a large loss in apples. Spinach stored at room temperature will lose all its vitamin C in a few days. Unrefrigerated handling of fresh fruits and vegetables in the market brings about large losses in vitamin C.

C. G. King stated that large numbers of the population at large are suffering from a deficiency of vitamin C. Where vitamins such as C and D must be administered, we cannot depend upon the diet. Vitamin C is easily destroyed and vitamin D is not widely distributed. In 50% of cases of scurvy, investigation proved that the mother had lied about the administration of vitamin C. The ascorbic acid content of the body tissues varies with the adequacy of vitamin C nutrition. Vitamin C is necessary for the action of the complements in immunity reactions. Evidence indicates that many humans are in a stage of vitamin C depletion which if present in the guinea pig would be sufficient to render the pig's teeth susceptible to injury by toxins. Doctor King stated that it would be advantageous to administer ascorbic acid in a citric acid solution to decrease destruction in the intestinal tract.

Vitamin D

Edwards A. Park stated that ten forms of vitamin D had been discovered, of which two, irradiated ergosterol and irradiated 7-dehydro cholesterol were of practical importance. Thirty-nine investigations have been reported of the results of administering various forms of vitamin D to infants, all the results not being in agreement. In these clinical observations it is hard to get enough children. Italian infants are susceptible to rickets while Jewish children are nearly immune. As nearly as one can determine, the vitamin D in irradiated ergosterol is as effective an antirachitic for the human as is the D in cod liver oil, rat unit for rat unit. In the human, the maximum incidence of rickets is at 4 months of age, and the administration of vitamin D should be started at 2 weeks of age. In oils, 800 to 1000 international units of vitamin D daily is the lower level of dosage for prevention of rickets. Though any amount of vitamin D which will prevent rickets will eventually cure the condition, we should produce a cure more rapidly. Premature infants may require from 5000 to 10,000 international units of vitamin D to prevent rickets. Vitamin D dispersed in milk is more effectual, unit for unit, than vitamin D in oils. Four hundred international units of vitamin D per quart of milk is better than 135 units. He is thoroughly convinced of the advantages of using milk as a source of vitamin D, provided the milk has been reinforced with a sufficient amount of the vitamin.

The administration of vitamin D should be continued throughout the growth period. It is harmless and certainly improves tooth nutrition. It should be administered to pregnant women, and to individuals who are deprived of being out of doors, such as miners and invalids. There are a number of conditions in the adult where vitamin D administration is indicated. If this does not affect the calcium and phosphorus content of the blood, its use is innocuous. Doctor Park advocated the use of vitamin D substances which also contain vitamin A, since it is just as easy to administer vitamin D plus vitamin A as vitamin D alone. In relation to the cost per unit of vitamin D, cod liver oil is cheapest, percomorph liver oils stand next, viosterol next, and irradiated milks are the most expensive.

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